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Chemical Characterization of Inbred Strain Mouse Milk. II. Total Fatty Acids and Fatty Acid Analyses.* (31810)

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Chemical analysis of inbred strain mouse milk is now possible through the development of a simple reliable milking apparatus and the recent progress in micro-analytical methods(1). A previous paper reported on certain gross compositional observations and amino acid patterns of milk from DBA/2J and C57BL/6J mice(2). The same 17 amino acids were present in both strains, but quantitative differences were evident especially in the free amino acid fraction. Since both strains were fed an essentially similar diet except for a small difference in fat content, the deviations in amino acids could be assumed to be due to the genotypic differences between the strains.

The purpose of this study was to characterize the fatty acid composition of mouse milk regarding number of different fatty acids, their levels, and possible differences between mouse genotypes.

Materials. The 2 inbred strains studied were DBA/2J and C57BL/6J. Both are non-agouti (gene symbols, *aa*), and they differ at the brown (*b*) and dilute (*d*) loci; C57BL/6J is wild type (+) at these 2 loci and DBA/2J is *bb dd*. F_1 hybrids, $B6D2F_1$, were obtained by mating C57BL/6J with DBA/2J females. $B6D2F_1$ females were backcrossed to DBA/2J males, yielding several kinds of offspring with respect to coat color. They were dilute black (*b*+ *dd*), brown (*bb d*+), and dilute brown (*bb dd*). The $B6D2F_1$ is identical with respect to coat color to the black backcross mice. Female offspring from each of the backcrosses were mated with DBA/2J males. The number of black backcross mice obtained was not sufficient to provide adequate milk for analysis. The milk was collected between the first and second week of lactation, and milk samples from 5 to 10 mice were pooled. Lactating mice were separated from their young for 16 hours, *i.e.*, overnight, and milked in the morning. The females had free access to food and water. The mouse diet contained 2% fiber, 19% protein from skim milk and wheat, and 11% fat from corn oil for all mice except DBA/2J whose diet contained only 8% fat. Ash content was 4.7%, calcium 1.0% and phosphorus 0.6%. The amounts of vitamins per kg were as follows: vit A 13640 USP, thiamine 8.2

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TABLE I. Fatty Acid Breakdown of Staley Corn Oil, in g/100 g of Total Fatty Acids.*

Fatty acid	Avg	Range
C^{16}	8.0	7- 8
C^{18}	3.5	3- 4
$C^{18:1}$	46.0	43-47
$C^{18:2}$	42.0	39-42
C^{20}	.5	trace-.5

* From A. E. Staley Manufacturing Co., Decatur, Ill., through courtesy of E. Morse, Emory Morse Co., Guilford, Conn.

mg, riboflavin 9.0 mg, vit E 133.1 mg, niacin 49.1 mg, and pantothenic acid 18.3 mg. The diets were made essentially according to the Morris formulations(3). Corn oil used in Morris formulations is produced by A. E. Staley Manufacturing Co., Decatur, Ill. and was found to have the fatty acid breakdown presented in Table I.

Methods. Analyses were performed on replicate samples, *i.e.*, 2 separate samples from each group of mice were collected and fatty acid composition determined. One ml of milk was extracted with petroleum ether following dilution with weak ammonia. The extracts were dried and the fatty acids transesterified by sealed tube incubation with 5 ml of 2% H_2SO_4 in methanol (v/v), at a temperature of 65°C for 6 hours. Following concentration of the esterification medium, H_2O was added and the fatty acid methyl esters extracted with petroleum ether. The extracts were then washed, dried under N_2, and made to 4 ml with hexane for gas liquid chromatography (GLC). The GLC conditions were as follows: (a) 183 cm × 0.32 cm polyglycol succinate ester, 5% on Diatoport S; (b) injection port-detector (flame ionization) at 220-230°C; (c) sample size, 3 μl; (d) He flow, 25 ml/min; (e) column temperature, 75°C isothermal through elution of methyl caprylate, thereafter to 190°C with programming at 5.6°C/min; (f) quantification of GLC peaks was made by integrator counts in comparison with fatty acid methyl ester standards.

Results. Pooling of milk samples was necessary because the yield of milk from individual lactating primiparous mice was insufficient for analysis. The backcross matings were made so as to produce animals with black, brown, or dilute brown coats and the milk from the backcrosses was pooled according to the coat color of the donors.

Table II lists the relative amounts of fatty acids contained in the milk. All 6 samples contained 14 different types. Eight were saturated and 6 were unsaturated fatty acids, of which 4 had one double bond and 2 were doubly unsaturated. Linoleic (18:2) and palmitic acid (C^{16}) occurred in greatest concentration followed by oleic acid (18:2) and these accounted for about 60% of the total fatty acid concentration. Oleic acid (18:2) was markedly lower in DBA/2J than in all the other milk samples. Also the values of fatty acids in the (C57BL/6J

TABLE II. Fatty Acid Composition of Mouse Milk of Different Inbred Strains and Their Hybrids, in Per Cent of Total Fatty Acid Concentration.

	C57BL/6J	DBA/2J	B6D2F$_1$	Backcross offspring (1)	Backcross offspring (2)	Backcross offspring (3)
Fatty acid	*aa*	*aa bb dd*	*aa b+ d+*	*aa b+ dd*	*aa bb d+*	*aa bb dd*
C^{10}	7.61 ± .54*	10.25 ± .24	7.53 ± .03	6.20 ± .17	6.66 ± .34	5.14 ± .37
C^{12}	12.50 ± .83	14.86 ±1.21	10.03 ± .04	10.35 ± .14	10.15 ± .33	8.09 ± .96
C^{14}	13.20 ± .13	14.22 ± .01	11.17 ± .36	11.06 ± .76	11.47 ± .48	9.99 ±1.02
C^{16}	22.27 ±1.23	22.26 ± .60	19.77 ± .38	21.67 ± .58	20.86 ± .33	20.99 ±2.03
$C^{16:1}$	1.85 ± .30	1.29 ± .48	.74 ± .55	1.29 ± .56	1.37 ± .75	7.61 ±5.23
C^{18}	1.77 ± .23	1.29 ± .30	2.07 ± .33	1.79 ± .24	2.36 ± .05	2.09 ± .40
$C^{18:1}$	16.29 ± .78	11.80 ± .37	19.20 ± .21	20.07 ± .25	20.33 ± .13	20.17 ±2.10
$C^{18:2}$	21.29 ± .37	20.62 ± .50	23.72 ± .89	23.43 ±1.65	23.33 ±2.49	22.07 ± .03
C^{20}	.86 ± .24	1.08 ± .54	.66 ± .26	.44 ± .09	.20 ± .09	.62 ± .39
$C^{20:1}$	.38 ± .06	.94 ± .25	1.66 ± .18	.92 ± .33	.81 ± .13	1.08 ± .66
$C^{20:2}$	.95 ± .28	1.02 ± .35	1.84 ± .19	1.31 ± .09	1.27 ± .08	.92 ± .07
C^{22}	.47 ± .14	.38 ± .11	.76 ± .21	.69 ± .01	.55 ± .10	.51 ± .22
$C^{22:1}$	.58 ± .03	.56 ± .03	.86 ± .30	.81 ± .06	.66 ± .07	.74 ± .44

C^8 was present in all samples as trace amount or less than 0.02 g/100 ml.
* Standard deviation.

TABLE III. Saturated and Unsaturated Fatty Acids in Mouse Milk in g/100 ml.

Strain	Saturated C^8 to C^{10}	Saturated C^{12} to C^{22}	Unsaturated C^{10} to C^{22}	Total fatty acids
C57BL/6J	.60 ± .14	3.97 ± .11	3.22 ± .08	7.79 ± 1.28
DBA/2J	.91 ± .11	4.74 ± .79	3.22 ± .53	8.86 ± 1.33
B6D2F$_1$	.79 ± .01	4.70 ± .78	5.08 ± .84	10.57 ± .25
BC$_1$	1.18 ± .07	8.72 ± 1.45	9.12 ± 1.52	19.02 ± 1.75
BC$_2$	1.16 ± .03	7.91 ± 1.31	8.30 ± 1.38	17.37 ± .37
BC$_3$	.90 ± .05	7.41 ± 1.23	9.23 ± 1.53	17.54 ± .29

× DBA/2J)F$_1$ did not fall in between those of the parents. None of the 6 milk samples contained linolenic or arachidonic acid. Table III presents data on the total fatty acid concentration and the fractions of saturated and unsaturated fatty acids. The total fatty acid concentration of the milk samples varied markedly. Approximately 50% of the fatty acids contained in the milk were saturated and the total of volatile acids (below C^{10}) was very small.

Discussion. All samples of the milk contained 14 fatty acids. There usually are over 20 in human and cow's milk but the amounts of fatty acids are readily affected by dietary changes(4). The source of fat in the mouse diet was corn oil, for which the main constituent fatty acids were oleic, linoleic, and palmitic acids (Table I). Linoleic, linolenic, and arachidonic acids are considered "essential" fatty acids for man, rats, and mice(5-7). The large amounts of linoleic (18:2), oleic (18:2), and palmitic (C^{16}) acids in all mouse milk samples were probably in part due to their high concentration in the corn oil. Likewise, the absence of linolenic and arachidonic acid in mouse milk is probably related to the lack of these fatty acids in corn oil(7). Whereas it might appear that mice are unable to synthesize them as well as other tri- and polyethenoid acids, there is no real reason to assume that mice cannot convert linoleic to arachidonic acid. Although the nutritional significance of linolenic or arachidonic acid for mice is not completely known, there is evidence that the linolenic acid requirement of the mouse is similar to that of the rat(8,9). As in the rat, there was no butyric acid present in mouse milk(6). Its absence might explain the lack of odor of curdled milk in the stomach contents of suckling rats and mice. There was less than 0.02 g/100 g of caprylic acid in mouse milk, whereas rat's milk contains 0.35 mg/100 ml(6). The $C^{20:1}$ fraction of mouse milk may be isomeric with methyl-ciseicosa-5-eonate because of their similar retention times. The $C^{20:2}$ fatty acid remains unidentified, but definitely is neither a C^{22} nor a $C^{20:1}$ acid. The $C^{22:1}$ fatty acid probably is erucic.

Despite apparent quantitative differences in fatty acid composition between the milk samples, genetic analysis proved unfeasible because: (1) grouping of backcross mice by coat color does not consider all of the large number of genes segregating in these mice; and (2) pooling of milk eliminated the possibility of detecting individual differences. Ideally, milk from *individual* backcross mice should have been analyzed. It appears questionable whether or not the two coat color genes control fatty acid levels because of the marked variation of values in the DBA/2J and the third backcross offspring both of which are dilute brown (Table II). Yet, if the fatty acid content of milk were totally diet dependent, then the concentration of oleic acid should be highest, which it is not, and the palmitic acid concentration would not be expected to be as high, as it was found to be. Therefore, the possibility of (a) genetic influence(s) upon fatty acid concentration exists. Such an indication derives also from the fact that milks from C57BL/6J and all crosses differ greatly from DBA/2J with respect to oleic acid concentration.

The total fatty acid content of mouse milk is very high compared to that of cow's milk. This high content is especially apparent in milk from the backcrosses. We have previously reported that mouse milk had a lipid and butterfat content several

times greater than that of cows. In milk of strain C57BL/6J, the lipid concentration was 41.6 ± 7.3%, and butterfat was 22.0 ± 3.7%(2).

Summary. Fatty acid composition of milk from DBA/2J, C57BL/6J, B6D2F$_1$ and 3 backcross types classified according to coat color (B6D2F$_1$ × DBA/2J) was obtained by gas liquid chromatography. The total of fatty acids in mouse milk ranged from 7.79 to 19.02 g/100 ml and 14 fatty acids were identified. Measureable quantities, 0.02 g/100 ml or more, of C^{10} to $C^{22:1}$ were present. Eight were saturated fatty acids and of the 6 unsaturated ones, 4 were singly unsaturated and 2 were doubly unsaturated. Linoleic, palmitic, and oleic acids occurred in highest concentration, probably because the dietary corn oil is especially rich in these acids. Linolenic acid and arachidonic acids were absent in milk, and are also lacking in corn oil. Although genetic analysis of fatty acid composition could not be done because of procedural difficulties in obtaining sufficiently large samples of milk from individual mice, the possibility of genetic influences upon fatty acid levels exists for reasons which are discussed.

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Cytosine Arabinoside Sensitivity in Actinobolin-Resistant *Streptococcus faecalis*: The Basis of a Utilitarian Microbiological Assay.* (31811)

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Cytosine arabinoside (1-β-D-arabinofuranosylcytosine hydrochloride) has been reported to inhibit a variety of experimental mammalian neoplasms *in vivo*(1-5). It also has antiviral activity: inhibition in cell culture of the DNA viruses of herpes simplex, B-virus, pseudorabies, vaccinia, swine pox and fowl pox, but lack of inhibition of several adenoviruses and RNA viruses has been reported(6-12). Cytosine arabinoside has been briefly reported to inhibit the anaerobic growth of *Escherichia coli*(13) but a definitive evaluation of the antimicrobial properties of this compound has not been published.

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Initial reports indicate that cytosine arabinoside has antileukemic activity in humans (14-20). The applicability of first order reaction kinetics of cell kill in leukemia and other tumors by antineoplastic agents(21) has emphasized the need to determine inhibitor concentration-time relationships in both experimental animals and humans. A microbiological assay for cytosine arabinoside has been developed and its utility demonstrated for biological samples.

Antimicrobial activity determinations. In a search for a potential assay microorganism, 325 microorganisms (bacteria, yeast and fungi) were tested for sentitivity to cytosine arabinoside using previously described methods(22) (see reference 23 for complete listing of the cultures used). Agar-diffusion