

Fatty Acids of the Pregnant Rat Uterus

Near the time of ovulation in women, the synthesis of endometrial triglycerides and sterol esters is altered dramatically^{1,2}. Fatty acids which are esterified to cholesterol and glycerol in human endometrium also change during the menstrual cycle³. However, with few exceptions^{4,5} uterine fatty acids have remained largely unexplored. Those results which have been reported indicate that endometrial fatty acids could be important to the developing blastocyst³. If this is so, the fatty acid population in the uterus might change markedly during early pregnancy. To investigate this possibility, a pilot experiment using rats was performed.

Six virgin female white rats (Holtzman Co.) were mated with males of proven fertility. Mating was observed and confirmed by the presence of sperm in the vaginal smear. At 1, 48, 72 and 120 h postcoitum, the females were sacrificed and the uteri removed and dissected free of surrounding tissues. The horns were slit longitudinally and incubated for 2 h in Hanks' solution with acetate-1-¹⁴C in an atmosphere of 95% O₂; 5% CO₂. (For the purposes of this paper, no reference will be made to the ¹⁴C data.)

After incubation, the uteri were removed and cleansed over filter paper. The lipids were extracted with repeated washes of chloroform-methanol (2:1, v/v). The extract was purified by changing the solvent to chloroform and removing precipitated materials by filtration⁶. Phospholipids were removed from neutral lipids by precipitation with dry acetone and MgCl₂ at 4°C followed by filtration⁷. Neutral lipids were separated into sterol esters, triglycerides, free sterols, and free fatty acids using a semiautomatic system and column chromatography⁷. Since the free sterol fractions obtained with this method contain diglycerides⁸, free sterol fractions were also subjected to fatty acid analysis. Following hydrolysis of the esterified fats and methylation, the methylated fatty acids from each lipid class were separated by gas liquid chromatography. The population distributions of the fatty acids from 23 fractions were then calculated.

Evaluation of the data failed to demonstrate any patterns of change related to time postcoitum. The fatty acids were also similar in the 4 lipid classes examined. Since no obvious changes in relation to class or time were seen, the data were pooled and analyzed for frequency of occurrence and percent of total population by Duncan's multiple range test.

Fatty acids C-10:0, 12:0, 14:0, 16:0, 16:1, 18:0, and 18:1 were detected with significantly greater frequency than were the other fatty acids (Table; all *p* values < 0.01). Interestingly, the odd-chain fatty acids 15:0 and 15:1 were present in 95% and 44%, respectively, of the samples analyzed.

The fatty acids which occurred most frequently generally accounted for the largest portion of the total population. Analysis of the population distribution of individual fatty acids indicated the following significant differences in relative amounts present: 16:0 > 18:1 > 16:1 > 18:0 > all other fatty acids (all *p* values < 0.01). Unsaturated acids, per se, accounted for about 38% of the total population. Of the unsaturated fatty acids, 16:1 and 18:1 occurred most frequently and accounted for about 84% of the total.

The lack of change after copulation in the population distribution of fatty acids agrees with the work of Goswami et al.⁵. These workers analyzed unsaturated fatty acids from mice uteri and concluded that 'It is unlikely that estrogen exerts any significant influence on metabolism of unsaturated fatty acids per se'.

DEALVAREZ et al.⁴ analyzed the total lipid (which included phospholipids) fatty acids in normal and malignant uterine tissues from women. They found long-chain unsaturated fatty acids like C-22:6 were present in malignant tissue but not in normal tissues. In their study, normal uterine tissues contained 30–60% of fatty acids population with carbon chain lengths of 14. By comparison, rat uterine tissues contained some 50% of the total population as 16:0 and 16:1. However, my analyses did not include fatty acids of phospholipids.

Population distribution of fatty acids

Carbon chain length	Frequency of occurrence in 23 samples	Average distribution pattern \pm SE
10:0	23	3.7 \pm 0.65
12:0	23	3.9 \pm 0.45
X-1	12	1.7 \pm 0.41
X-2	7	0.8 \pm 0.37
X-3	2	0.4 \pm 0.35
14:0	23	6.5 \pm 0.44
14:1	6	0.8 \pm 0.35
15:0	22	4.8 \pm 0.40
15:1	10	1.6 \pm 0.42
15:2	1	0.2 \pm 0.24
16:0	23	33.1 \pm 0.90
16:1	23	14.3 \pm 0.98
16:2	4	0.4 \pm 0.20
17:0	4	0.6 \pm 0.28
18:0	22	9.8 \pm 1.04
18:1	22	17.5 \pm 1.54
18:2	9	2.9 \pm 1.03

Those fatty acids designated 'X' were not identified but were eluted between fatty acids 12:0 and 14:0.

Zusammenfassung. Die Untersuchung über die Fettsäure-Zusammensetzung der Ratten-Uteruslipide während der Gravidität ergab ein unverändertes Verbreitungsmuster bei den Hauptfettsäuren der neutralen Lipide.

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¹ J. R. BEALL, Master Thesis, Univ. Oklahoma, Norman, Okla., 1-47 (1964).

² J. A. MERRILL and N. T. WERTHESSEN, *Am. J. Obstet. Gynec.* **96**, 619 (1966).

³ V. H. BURGER and N. ZOLLNER, *Arch. Gynaek.* **206**, 107 (1968).

⁴ R. R. DEALVAREZ, H. CASTELLANO, D. O'LEARY and F. JAHED, *Am. J. Obstet. Gynec.* **104**, 230 (1969).

⁵ A. GOSWAMI, A. B. KAR and S. R. CHOWDHURY, *J. Reprod. Fertil.* **6**, 287 (1963).

⁶ J. R. BEALL and N. T. WERTHESSEN, *J. Endocr.*, in press (1971).

⁷ N. T. WERTHESSEN, J. R. BEALL and A. T. JAMES, *J. Chromatogr.* **46**, 149 (1970).

⁸ From the Institutes of Health Sciences, Brown University, Providence, R.I., USA. Supported in part by NIH grant No. HD-02599.