大阪公立大学
Osaka Metropolitan University
学術情報リポジトリ

Polyunsaturated Fatty Acids in the Rat Placenta

メタデータ	言語: eng 出版者: 公開日: 2009-08-25 キーワード (Ja): キーワード (En): 作成者: ICHIKAWA, Shigetaka, YAMADA, Jiro メールアドレス: 所属:
URL	https://doi.org/10.24729/00009536

Bull. Univ. Osaka Pref., Ser. B.
大阪府立大学紀要，農学・生物学 Vol. 15, 1964

Polyunsaturated Fatty Acids in the Rat Placenta

By

Shigetaka ICHIKAWA and Jiro YAMADA

Department of Veterinary Science, College of Agriculture

(Received August 30, 1963)

A lipid-soluble oxytocic substance isolated from the parturient human placenta has been identified to be arachidonic acid[1,2]. With the view of obtaining some information about the responsibility of arachidonic acid for the onset of labor, the present study was designed to determine the pattern of changes in the concentrations of polyunsaturated fatty acids (PUFA) in the rat placenta during the course of normal pregnancy.

Materials and Methods

Wistar strain female rats fed on the F.R.L. Breeder diet[3], were placed with males in the evening and vaginal smears were taken on the following morning. The day on which spermatozoa were observed in the smear was regarded as the first day of pregnancy. These rats usually delivered youngs in the period between the evening of the 22nd day and the morning of the 23rd day of pregnancy. The gravid rats, weighing 200 - 300 g., were presented for the examinations on the 16th, 18th, 20th or 22nd day of pregnancy. As soon as the animals were bled by cardiac puncture using sodium oxalate as an anticoagulant under ether anesthesia and killed by exsanguination, placenta and liver were excised and cooled in ice.

EXTRACTION OF LIPIDS

All extractions of lipids in the plasma, placenta and liver were carried out in duplicate for each animal, except for the case of placenta on the 16th day of pregnancy wherein the amount of tissue was not sufficient for the need of duplicate trials. All procedures were performed under nitrogen.

All solvents were redistilled. Methanol was freed from aldehydes by distillation from KOH pellets. Chloroform used for extraction contained 1.5 % of methanol as a preservative, and chloroform used for chromatography was freed from methanol and freshly redistilled before each service.

Plasma lipids. Lipids in 1 ml. of plasma were extracted with 20 ml. of chloroform-methanol (exactly 2 : 1 v/v) and dialyzed against 20 ml. of distilled water following the procedure described by ALBRINK[4]. The mean amount of clear chloroform extract obtained was 12.75 ml. Two 2-ml. portions of the extract were used for lipid phosphorus determination. Five ml. of the chloroform extract was pipetted into a 10 ml. volumetric flask. The solvent was evaporated under the nitrogen stream until just before dryness. For the purpose of removal of the trace of chloroform, 2 ml. of methanol was added twice to the residue and evaporated each time. Finally petroleum ether was added to the mark. The solution was used for the determination of PUFA.

Tissue lipids. Lipids of placenta and liver were extracted and fractionated as follows: A portion, approximately 1 g., of placenta or liver was weighed and homogenized in about 20

ml. of chloroform-methanol in a Potter-Elvehjem apparatus. The tube was then stoppered and allowed to stand at room temperature overnight. The homogenized extract was filtered off through ether-extracted filter-paper into a volumetric flask. The residue was washed repeatedly with several portions of chloroform-methanol and the combined filtrate was made up to 40 ml. Four 1-ml. portions of the chloroform-methanol extract were used for the determinations of free and total cholesterol. Exactly 20 ml. of the chloroform-methanol extract was pipetted into a 50 ml. centrifuge tube, and dialized against the equal volume of water as in the case of plasma lipids. The amount of chloroform extract obtained was 12.55 ml. on the average. Ten ml. of the chloroform extract was pipetted into a round-bottom centrifuge tube, and chloroform was evaporated until just before dryness. The residual lipids were dissolved in about 2 ml. of light petroleum and charged on a column (1.4 g. of silicic acid and 0.7 g. of Hyflo Super-Cel). The chromatographic separation of the lipids was carried out by the method described by BORGSTRÖM[5,6]. In the preliminary separation study, the concentration of tetraenoic acid in the ester-cholesterol fraction had been less than 10 per cent of that in the neutral-fat fraction at each gestation stage. The lipids were, therefore, separated into two fractions by elution with 70 ml. of chloroform and 40 ml. of methanol successively. These volume of solvents and the amount of silicic acid were sufficient for complete separation of each lipid fraction as shown in figure 1.

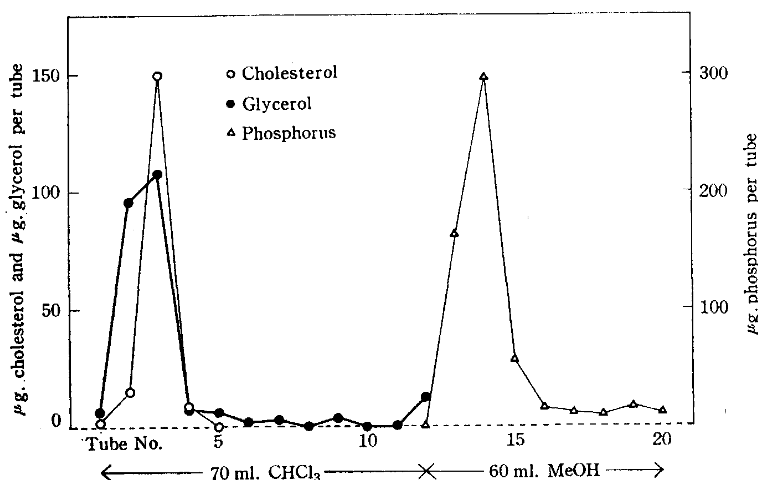

Fig. 1. Elution pattern of lipids from 1.4 g. of silicic acid with chloroform and methanol. The lipids were prepared from 0.985 g. of hepatic tissue of a pregnant rat (18th day) following the procedure described in the text. Flow rate, 1 ml./minute. Volume of eluate in each tube was 6 ml.

The chloroform eluate was referred to neutral-fat fraction, since most of PUFA in this eluate would probably be present in the form of neutral fat, although ester cholesterol was included in this eluate. Lipids in 10 ml. of the chloroform eluate and phospholipids in 5 ml. of the methanol eluate (phospholipid fraction) were dissolved into 10 ml. of light petroleum respectively after complete removal of chloroform or methanol. The light-petroleum solutions were used for the determination of PUFA. Four 2-ml. portions of the chloroform eluate were used for the determination of glycerol. Three portions of 1 or 2 ml. of methanol eluate were used for the determination of lipid phosphorus.

ANALYSES.

Phosphorus was determined by the method of STEWART and HENDRY[7], cholesterol by the method of SPERRY and WEBB[8] and glycerol by the method of VAN HANDEL and ZILVERSMIT[9]. PUFA concentrations in plasma and tissue lipids were measured by the alkaline isomerization method described by HOLMAN and HAYES[10].

Results

Figure 2 shows changes in the concentrations of phospholipids and PUFA in plasma during the period between the 16th and 22nd day of pregnancy. The concentration of each polyenoic acid in plasma increased steadily from the 16th to the 20th day with their relative proportions unchanged, and remained constant thereafter to term. Dienoic and

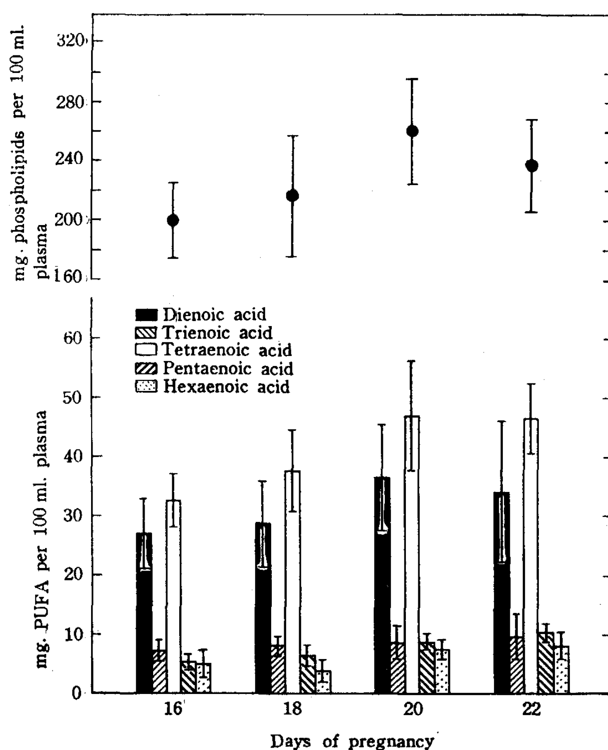

Fig. 2. Concentrations of phospholipids and PUFA in the pregnant rat plasma. Mean and standard deviation of 8 animals in each age group except in the 16th-day group (7 animals).

tetraenoic acid in the plasma lipids composed 33% and 43% respectively of total PUFA on the average during the period. The changes in the concentrations of PUFA closely paralleled the changes in phospholipids.

The concentrations of PUFA in the phospholipid fractions in both placental and hepatic tissues did not show any remarkable changes during the period examined (Fig. 3). The concentrations of arachidonic acid in this fraction were about 450 mg. per 100 g. of fresh hepatic tissue (47% of the total PUFA) and about 155 mg. per 100 g. of fresh placental tissue (53% of total PUFA). These values of arachidonic acid were extremely higher than those of the other polyenoic acids in the phospholipid fraction. The concentrations of phospholipids in both liver and placenta did not show any significant changes during the

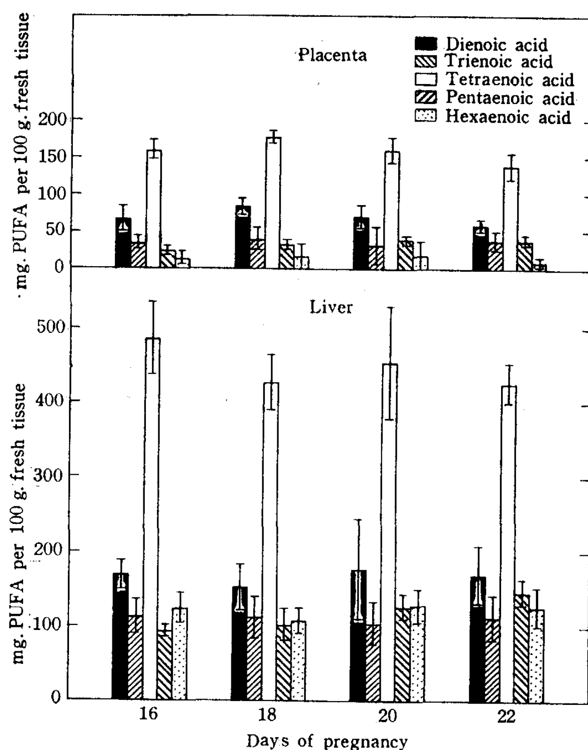

Fig. 3. Concentrations of PUFA in the phospholipids fractions separated from the placental and hepatic tissues of pregnant rats. Mean and standard deviation of 8 animals in each age group.

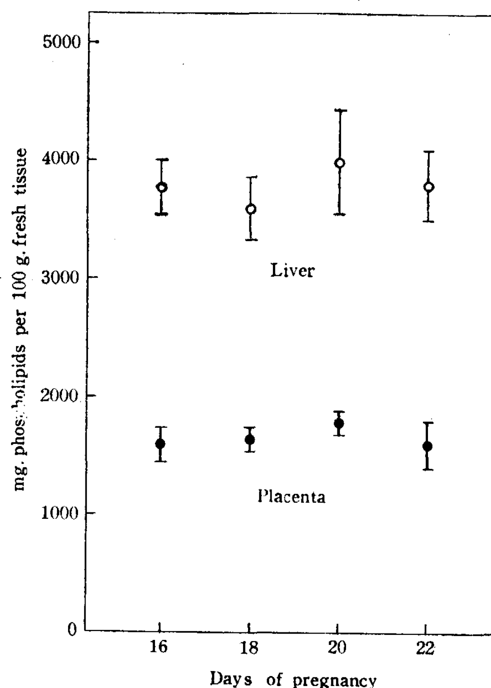

Fig. 4. Concentrations of phospholipids in the placental and hepatic tissues of pregnant rats. Mean and standard deviation of 8 animals in each age group.

period as those of PUFA in the phospholipid fraction (Fig. 4).

Figure 5 shows the changes in the concentrations of PUFA in the neutral-fat fractions of both placental and hepatic tissues. In the neutral-fat fraction of the liver, the concentrations of dienoic acid were always higher than those of arachidonic acid at any stage of the late gestation period and the concentrations of arachidonic acid remained at a constant level of about 17 mg. per cent without considerable variation. In the placental tissue, the concentrations of arachidonic acid in the neutral-fat fraction continued to rise from 36 mg. per cent on the 16th day to 45 mg. per cent on the 20th day of pregnancy and the values tended to fall somewhat at term, while the values of dienoic acid in this fraction exhibited the maximum value on the 18th day and decreased thereafter. As the result, the relative proportions of arachidonic acid to dienoic acid in the neutral-fat fraction of the placenta changed reversely with the approach of the term. The concentrations of arachidonic acid in the neutral-fat fraction of placenta were about two and a half times as high as those in the liver, in spite of the lower glycerol level in the neutral-fat fraction of placenta than that of liver (Fig. 6). The concentrations of glycerol in the neutral-fat fraction of placenta decreased remarkably at term. Therefore, the mole ratio of arachidonic acid to glycerol in this fraction rose from around 0.3 on the 20th day to 0.4 on the 22nd day of pregnancy (Fig. 7).

Figure 8 shows the changes in the concentrations of free and ester cholesterol in both placenta and liver. Free cholesterol continued to rise in concentration from the 16th day until the termination of pregnancy, while ester cholesterol remained constant during this period.

The content of water in the hepatic tissue was about 71% throughout the period

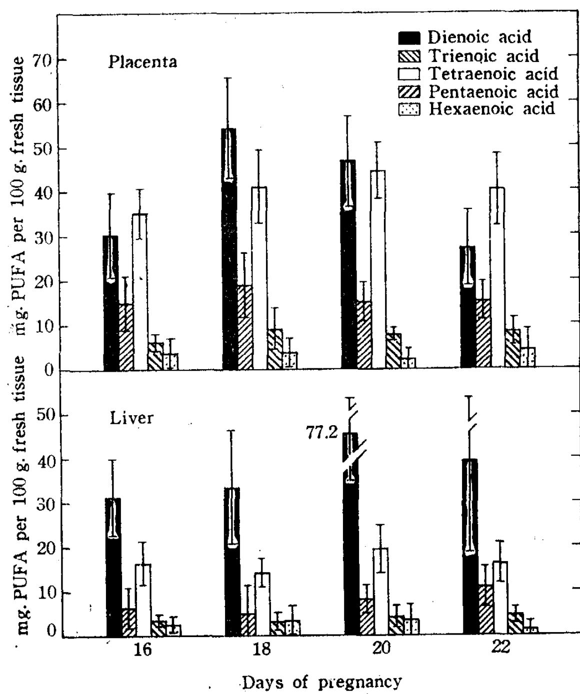

Fig. 5. Concentrations of PUFA in the neutral-fat fractions separated from the placental and hepatic tissues of pregnant rats. Mean and standard deviation of 8 animals in each age group.

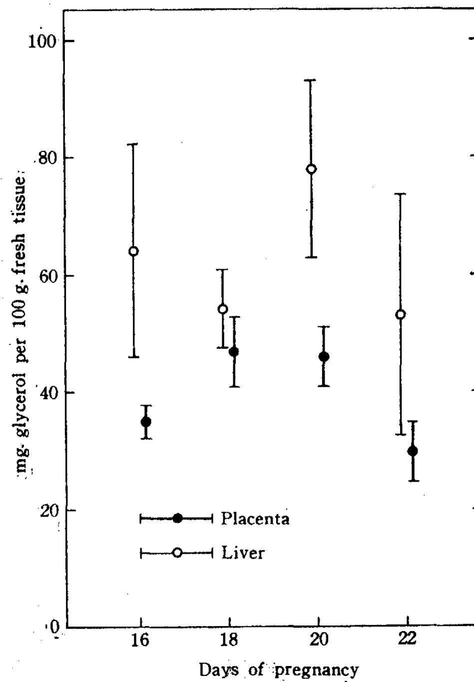

Fig. 6. Concentrations of glycerol in the neutral-fat fractions separated from the placental and hepatic tissues of pregnant rats. Mean and standard deviation of 8 animals in each age group.

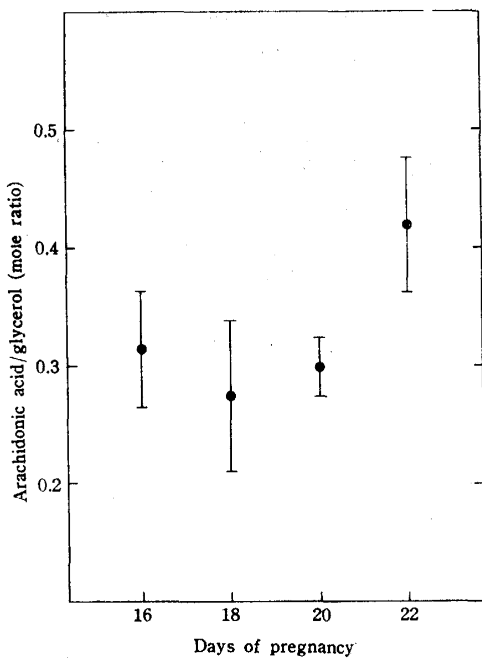

Fig. 7. Mole ratios of arachidonic acid to glycerol in the neutral-fat fractions from the rat placenta. Mean and standard deviation of 8 animals in each age group.

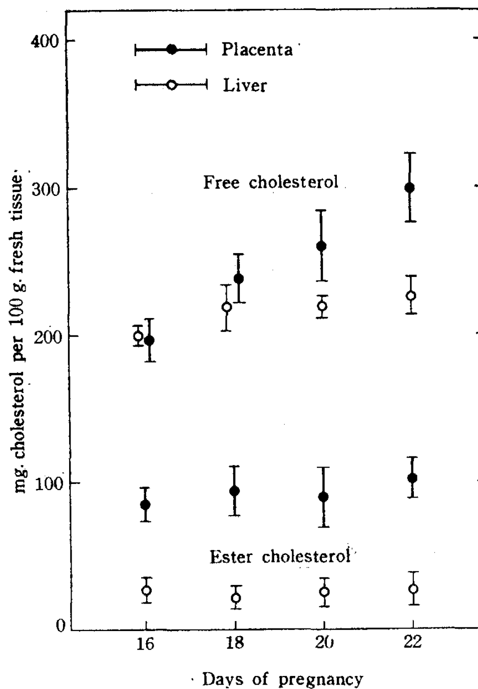

Fig. 8. Concentrations of free and ester cholesterol in the placental and hepatic tissues of pregnant rats. Mean and standard deviation of 8 animals in each age group.

Table 1. Water content in the placental and hepatic tissues of pregnant rats.

Days of pregnancy	PLACENTA		LIVER	
	No. of rats	Water content, % Mean±S.D.	No. of rats	Water content, % Mean±S.D.
16	9	85.3±0.44	10	71.5±1.01
18	6	84.3±0.28	8	71.6±1.04
20	12	83.9±0.38	12	71.5±1.42
21	4	83.6±1.00	4	71.4±1.79
22	8	83.0±0.99	10	70.8±1.68

examined. In the placental tissue the water content continued to decrease during the period, but at a very small rate (Table 1).

Discussion

The concentrations of phospholipids and PUFA that compose the phospholipids remained fairy constant in the placenta during the gestation period examined. These results indicate that the phospholipids, including their PUFA, occur in the placenta as a portion of the structural elements of the organ.

Arachidonic acid in the neutral-fat fraction in the placenta, although its concentration was about one fourth of that in the phospholipid fraction, exhibited an attractive pattern. The concentration of arachidonic acid in the neutral-fat fraction of placenta increased steadily as the end of pregnancy approached. The mole ratio of arachidonic acid to glycerol in the neutral-fat fraction of placenta was more than five times as high as the ratio in the same fraction of liver, and significantly increased at term. The values of the ratio are incorrect to the extent that some amounts of arachidonic acid derived from ester cholesterol contribute to the concentrations of the acid. In the preliminary experiment, however, the concentrations of arachidonic acid in the ester cholesterol fraction had been less than 10% of those in the neutral-fat fraction at each gestation stage. Moreover, the concentrations of ester cholesterol in the placenta remained constant during the period. Accordingly the changes in the mole ratio exhibit a significant fluctuation of the components of the neutral fat in the placenta. Relatively little is known about the physiological function of neutral fat apart from the fact that it is a reserve source of calories in the body. If arachidonic acid in the placental tissue should play a role as an oxytocic substance on the onset of labor, these results will suggest that it might be derived not from phospholipids but from neutral fat.

Summary

Changes in the concentrations of polyunsaturated fatty acids (PUFA) in the plasma, placenta and liver of the pregnant rats during the late pregnancy (16th - 22nd day) were studied.

PUFA levels in the plasma increased until the 20th day of pregnancy and remained at a constant level from then to term. The principal components of plasma PUFA were tetraenoic (43% of total PUFA) and dienoic acid (33% of total PUFA). PUFA in the phospholipids of both placenta and liver did not show any attractive changes in concentration during the period. Arachidonic acid composed about a half of the total amount of PUFA in the phospholipids of these organs. The concentrations of arachidonic acid in the placen-

tal neutral fat were about 2.5 times as high as those in the hepatic fat, and the mole ratio of arachidonic acid to glycerol in the neutral-fat fraction of placenta significantly increased at term.

References

1) ICHIKAWA, S. *Am. J. Physiol.* **198**: 1094, 1960.
2) ICHIKAWA, S. and J. YAMADA. *Am. J. Physiol.* **203**: 681, 1962.
3) HAWK, P. B., B. L. OSER and W. H. SUMMERSON. *Practical Physiological Chemistry*, 12th ed. London: Churchill, 1952, p. 1272.
4) ALBRINK, M. J. *J. Lipid Research*, **1**: 53, 1959.
5) BORGSTRÖM, B. *Acta physiol. Scandinav.* **25**: 101, 1952.
6) BORGSTRÖM, B. *Acta physiol. Scandinav.* **25**: 111, 1952.
7) STEWART, C. P. and E. B. HENDRY. *Biochem. J.* **29**: 1683, 1935.
8) SPERRY, W. M. and M. WEBB. *J. Biol. Chem.* **187**: 97, 1950.
9) VAN HANDEL, E. and D. B. ZILVERSMIT. *J. Lab. Clin. Med.* **50**: 152, 1957.
10) HOLMAN, R. T. and H. HAYES. *Anal. Chem.* **30**: 1422, 1958.