BBA 63358

Plasma lysolecithinase activity in pregnancy*

VIKROT[1,2] has demonstrated a progressive fall in the plasma concentration of lysolecithin in pregnant women and rats during most of the gestational period. EISENBERG, STEIN AND STEIN[3] confirmed this finding in rats and related the phenomenon to their observation that plasma lysolecithin is rapidly taken up by the placenta and further esterified to lecithin by the fatty acyl-CoA:lysolecithin fatty acyltransferase mechanism[4].

We are reporting another change in pregnant rhesus monkeys (*M. mulatta*) that may partly account for this depression in plasma lysolecithin concentrations: a 20-fold increase in lysolecithinase activity (lysolecithin acyl-hydrolase, EC 3.1.1.5) of plasma and high lysolecithinase activity in the placenta compared to other tissues.

Female rhesus monkeys that had been pregnant for known periods (determined from controlled mating periods) were bled from an arm vein. The blood was immediately chilled and the plasma harvested after centrifugation for 10 min at 0° and 1000 × *g*. Aliquots were added to chloroform–methanol (2:1, v/v) and the extracts were used for determination of lysolecithin concentrations. Other aliquots were used to determine plasma lysolecithinase.

Lipid extracts of plasma were prepared as previously described[5] and subjected to thin-layer chromatography in the system chloroform–methanol–water (62:37:7, by vol.). Lysolecithin concentrations were determined by a char-densitometric procedure similar to that described by PRIVETT *et al.*[6] but modified to use NH_4HSO_4 (ref. 7) as the charring reagent. This procedure was shown to reliably measure as little as 1 μg of lysolecithin. [^{14}C]Lysolecithin was prepared by incubation of [1-^{14}C]palmitate with a 1000 × *g* supernatant preparation of rat liver homogenate, subjecting the isolated lecithin to lecithinase A (EC 3.1.1.4), and purifying the lysolecithin by thin-layer chromatography. Lysolecithinase activity was determined by incubating plasma or 1000 × *g* cell-free preparations of tissue homogenates (maternal liver and placenta) with labeled lysolecithin. Reactions were terminated by the addition of chloroform–methanol (2:1, v/v) *plus* carrier lysolecithin. Substrate and products were separated by thin-layer chromatography and radioactivity was measured by scintillation counting. Quantities of lysolecithin hydrolyzed were calculated from the product of endogenous *plus* added lysolecithin concentrations in the plasma at the beginning of incubations and the fraction of radioactive fatty acid liberated. Blank values were obtained from incubations of plasma which had been heated to 58° for 30 min.

Table I shows the changes in plasma lysolecithin concentrations in rhesus monkeys during pregnancy. By day 60–89 of gestation, the concentration of lysolecithin was below that of nonpregnant females or of males (significance of difference between all pregnant and nonpregnant monkeys: $P < 0.001$). Plasma lysolecithin concentrations of nonpregnant females did not differ from those of males. Likewise, no apparent difference was seen between the plasma from females at the follicular stage of the ovarian cycle and that from females at the luteal stage. Within a few days after parturition, lysolecithin concentrations returned to levels seen in nonpregnant

* Publication No. 335 from the Oregon Regional Primate Research Center, supported in part by grant FR 00163 from the National Institutes of Health.

TABLE I

THE CONCENTRATION OF LYSOLECITHIN AND LYSOLECITHINASE ACTIVITY IN PLASMA OF SEVERAL GROUPS OF RHESUS MONKEYS

Data represent mean ± S.E.

Group	*Number*	*Lysolecithin concn. (nmoles/ml)*	*Number*	*Lysolecithinase activity (nmoles/h per ml)*
Males	10	212.3 ± 34.6	2	1.76
Females, nonpregnant	10	212.8 ± 18.2	15	1.47 ± 0.192
Females, pregnant:				
35–165 days	50	116.1 ± 9.9	18	26.16 ± 4.01
35– 59 days	12	182.2 ± 17.7		
60– 89 days	11	147.0 ± 23.4		
90–149 days	13	87.8 ± 8.6		
150–165 days	14	61.4 ± 4.4		
2 days postpartum	3	179.6		
Term fetus (about 160 days)	4	129.2	2	1.54
Newborn	3	139.4		

animals. Fetal and newborn rhesus monkeys had higher plasma lysolecithin values than did their mothers.

1-[1-^{14}C]palmityllysolecithin was hydrolyzed to a limited extent by plasma of males, nonpregnant females and fetuses. On the other hand, plasma from pregnant rhesus monkeys actively stimulated release of [1-^{14}C]palmitic acid from lysolecithin (Table 1). This activity was largely inhibited (> 90%) by heating the plasma for 30 min at 58° (ref. 8). Free fatty acids and lysolecithin were the main radioactive compounds although detectable radioactivity above that for the heated controls was present in the lecithin fraction. The marked increase in lysolecithinase activity, which occurred by the 42nd day of pregnancy, preceded a significant depression of plasma lysolecithin concentrations.

Cell-free preparations of placenta contained much more lysolecithinase activity (582 nmoles/h per mg protein) than did corresponding fractions of maternal liver (88 nmoles/h per mg protein). When we added linoleyl-CoA to these tissue preparations, significant quantities of lysolecithin were also esterified to lecithin (placenta, 178 and liver, 158 nmoles/h per mg protein). Both the tissue lysolecithinase activity and linoleyl-CoA:lysolecithin fatty acyltransferase activities[4] were heat-labile.

We obtained further support for the hypothesis that the placenta is an important source of the plasma lysolecithinase activity from the experiment shown in Fig. 1. The plasma lysolecithinase level rapidly declined toward levels seen in nonpregnant controls when the fetus and placenta were removed by cesarean section. When only the fetus was removed and the placenta was allowed to remain *in situ*[9], the plasma lysolecithinase activity remained elevated. After removal of the retained placenta, the enzyme activity then rapidly declined in a pattern similar to that seen when the placenta and fetus were removed together. Appropriate controls were used to establish that the changes in lysolecithinase activity were not a result of anesthesia, surgery, or fasting.

Although lysolecithinase activity has been reported in several tissues[10], there are conflicting reports about its presence in placenta[11,12] of other species. The hydrolysis

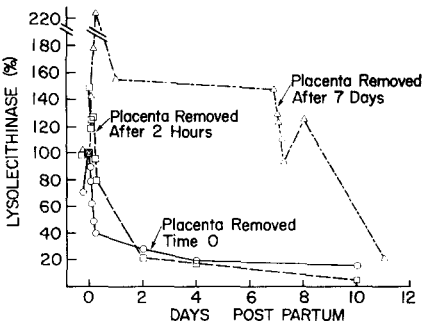

Fig. 1. The effect of removing by cesarean section the fetus and placenta at time 0 (○—○), the fetus at time 0 and the placenta after 2 h (□ – – –□); and the fetus at time 0 and the placenta after 7 days (△—· – ·—△) on the plasma lysolecithinase activity of the mother. The activities at time 0 (cutting of umbilical cord) were taken as 100%.

of lysolecithin by rhesus monkey placenta is much more active than its esterification even in the presence of added linoleyl-CoA. Esterification of lysolecithin predominated somewhat over its hydrolysis in cell-free homogenates of other tissues tested with linoleyl-CoA (liver, aorta, endometrium, and myometrium). Thus an important mechanism responsible for lowering plasma lysolecithin concentrations in pregnancy may be an increased hydrolysis of lysolecithin by enzymes of placental origin acting in the placenta or in plasma. Although we have not yet investigated lysolecithinase activity in pregnancies of less than 42 days duration this is substantially earlier than a viable fetus can be demonstrated by X-ray. The possible relevance of this enzyme activity to a test of human pregnancy is of considerable importance but remains to be established.

These results indicate that plasma lysolecithinase activities of pregnant rhesus monkeys are nearly 20 times greater than those of nonpregnant animals and that pregnant monkeys also have depressed plasma lysolecithin concentrations. This enzyme is probably derived from the placenta.

Supported by grant No. HE 09744-4, the National Heart Institute and Public Health Service grant No. 01901.

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Received August 9th, 1968