Effect of Pregnancy and Lactation on Triglycerides of Very-Low-Density Lipoproteins of Rat Plasma

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ABSTRACT
Triglyceride in the blood of rats increased during pregnancy, decreased to control during lactation, and increased again on weaning. The triglyceride content of the very-low-density lipoproteins (d<1.006 g/ml) changed in parallel with that of the plasma, and its magnitude indicated that it was chiefly responsible for the transport of triglycerides in the blood. These changes were accompanied by changes in the electrophoretic pattern of the lipoproteins of rat plasma, but no such changes were observed in lipoproteins of ovine and caprine serum.

INTRODUCTION
In a study of lipoproteins of rat serum during pregnancy and lactation, Bosch and Camejo (2) reported that the concentration of the triglycerides of the very-low-density lipoproteins (VLDL) increased during pregnancy and that elevated triglycerides in this lipoprotein persisted during lactation, up to 3 wk postpartum. This result was difficult to reconcile with the low concentration of plasma triglycerides in lactating rats reported by Hamosh et al. (5) and with our own preliminary observations (unpublished) of the lipoprotein patterns on gradient polyacrylamide gel electrophoresis. Therefore, we have re-examined the triglyceride concentrations in plasma and VLDL of rats during pregnancy, lactation, and after weaning.

Unlike the rat, ruminants do not exhibit hyperlipemia of pregnancy (13) although changes in the electrophoretic pattern of bovine serum lipoproteins have been observed in pregnancy and lactation (9). We have compared the electrophoretic patterns of circulating lipoproteins of rats with those of sheep and goats during pregnancy and lactation.

MATERIALS AND METHODS
Animals
Hooded Norway rats from the Institute colony were used. The animals were allowed food (Spillers small animal diet) and water ad libitum and were 3 to 3.5 mo old at mating. The procedure for mating has been described (12). For triglyceride estimation, the following animals were studied: a group of six unmated animals; groups of five animals at 7 and 21 days of pregnancy and at 14 days lactation; and groups of six animals at 16th day of pregnancy, 2nd day of lactation, and on the 3rd day after removing the young. In the last group, the young were removed on the 21st day after birth. For electrophoresis, different groups if five rats were studied. The groups were at the same stages of pregnancy and lactation as for triglyceride estimation with the exception of the rats pregnant 15 and 20 days. The rats were killed by cervical dislocation, and blood was sampled by heart puncture. The blood was prevented from clotting by the addition of EDTA (disodium salt, 1 mg/ml) and the plasma separated by centrifugation (1000 x g for 30 min).

Jugular blood was obtained from four Clun Forest sheep and from one British Saanen goat during pregnancy and early lactation and from two other goats nonpregnant and in late lactation. Serum was obtained as described (3).

Isolation of Very-Low-Density Lipoproteins
Plasma (.9 ml) in a 2.5 ml polycarbonate centrifuge tube was overlaid carefully with .9 ml of a solution of NaCl (.9% wt/vol) and centrifuged for 20 h at 12 C in the 10 x 10 ml angle rotor of an MSE Superspeed 50 ultracentrifuge at 157,000 x g (average). The upper half

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of the solution which contained the VLDL was removed for analysis.

**Estimation of Triglycerides**

This was by the method of Ryan and Rasho (11) except that 1 ml samples of plasma or VLDL were extracted and phospholipids were removed with 3 g of Florisil (1). Student’s t test was used to calculate statistical significances of the differences of the mean plasma triglyceride concentrations from that of nonpregnant control rats.

**Gradient Polyacrylamide Gel Electrophoresis**

Samples of serum or plasma (.2 ml) were pre-stained with a saturated solution of Sudan Black B in propylene glycol (.04 ml) (7) and the lipoproteins separated by electrophoresis on 3 to 11% polyacrylamide gels as in (3).

**RESULTS AND DISCUSSION**

The increase in concentration of triglycerides of rat plasma during pregnancy, decrease to control during lactation, and rise on weaning (Fig. 1), which agrees with the results of Hamosh et al. (5), were paralleled by corresponding changes in concentration of VLDL triglycerides (Fig. 1). The finding that the concentration of VLDL triglycerides was low during lactation was contrary to an earlier report (2) but is expected from the low triglyceride concentration of plasma in lactation, if, as shown by the proportion of the total plasma triglycerides which they carry, VLDL are chiefly responsible for the transport of triglycerides.

The rapid fall in concentration of plasma triglyceride just before parturition which has been reported (5, 8) was not observed here, but there is a fairly wide range of values for the 21-day-pregnant rat (2.6 to 7.6 μmol/ml), and the lower values may be because some animals were close to parturition. The fact that the 4.7 times increase in the plasma triglyceride concentration of rats during pregnancy reported by Ekholm et al. (4) was not statistically significant is a further demonstration that the concentration of plasma triglyceride can vary widely immediately before parturition.

The hypertriglyceridemia of pregnancy has been attributed to diminished uptake of triglyceride fatty acids by adipose tissue (8), and recently it has been suggested that hepatic lipogenesis, which increases markedly during pregnancy and lactation (12), also may play a part (6, 10). It has been suggested that the hypertriglyceridemia disappears in lactation because of the high concentration of mammary lipoprotein lipase, an enzyme which is involved in the uptake of triglyceride by the mammary gland, but that it reappears on weaning when the lipoprotein lipase concentration is decreased (5).

On electrophoresis of rat plasma lipoproteins, the intensity of the VLDL band relative to those of the rest of the plasma lipoproteins...
FIG. 2. Electrophoresis of typical rat plasma lipoproteins (prestained with Sudan Black B) on gradient polyacrylamide gels (3). Nonpregnant control, C; pregnant for 7, 15, and 20 days, 7P, 15P, and 20P; lactating for 2 and 14 days, 2 L and 14 L; weaned for 3 days, 3W. The very-low-density lipoprotein band is immediately below the top of the gel. (Fig. 2) changed markedly during pregnancy and lactation, in parallel with changes in triglyceride content (Fig. 1). For comparison, typical electrophoretic patterns of ovine and caprine serum lipoproteins are in Fig. 3. They showed no appreciable changes during pregnan-
cy and lactation. The changes reported by Raphael et al. (9) in the electrophoretic pattern of bovine serum lipoproteins affected mainly a low density alpha lipoprotein, which we have not observed in ovine or caprine serum (3).

The changes in intensity of bands corresponding to lipoproteins other than VLDL in rat plasma may not be due to changes in concentration. Unpublished experiments (Stead and Welch) have shown that when the standard staining procedure is used (7), lipemic sera do not stain as intensely as expected from their lipid content. However, the relative intensities of the bands are correct even though the intensity of each one is less than expected. It seems possible that in late pregnancy lipoproteins other than VLDL may not change in concentration but that the increase in VLDL lipid results in each of the bands having a reduced intensity. Since we primarily were interested in the VLDL, we have not investigated this aspect further, but it could best be resolved by isolation and analysis of the other lipoproteins.

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