It is known that cases of high anorectal atresia[11] and cases of Hirschsprung disease[12] may occasionally show the reflex of a similar pattern postoperatively, and it may be due to exaggeration of the normal intestinal intrinsic reflex[13] or to compensatory participation of spinal nerves[14]. Anyway, the phenomenon is different from the original rectosphincteric reflex, which is essentially a rectoanal intrinsic one.

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Cholesterol synthesis and related enzymes in rat liver during pregnancy

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Summary. During pregnancy the synthesis of cholesterol and the activity of 3-hydroxy 3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase fell markedly before parturition; HMGCoA synthase activity was low during pregnancy and fell again immediately before delivery while acetoacetyl-CoA-thiolase was always low and constant.

Important metabolic and hormonal changes occur during pregnancy to adapt maternal tissues to fetal growth[1]. Pregnancy is known to have far-reaching effects on lipid metabolism in several species[2-4]. Some results are available on fatty acid and total lipid synthesis and content in rat liver and adipose tissue during pregnancy[5], but little is known about hepatic cholesterogenesis and related enzyme activities which in many systems are modulated by several factors such as hormones and lipids.

In the present study we investigated in rat liver the pattern of acetate incorporation into cholesterol and the behavior of aceto-acetyl-CoA-thiolase, HMG-CoA-synthase and HMG-CoA-reductase activities from the last period of pregnancy up to birth.

Materials and methods. Female Wistar rats weighing 250 g and maintained with a standard diet ad libitum were exposed to a male for 24 h and pregnancy was diagnosed on the basis of the appearance of vaginal plug and checked by fetus weight and length. At times ranging from the 16th to the 22nd day of gestation the animals were sacrified after i.p. injection of anesthetic (Farmotal, Farmitalia, 20 mg/100 g b.wt). Then the livers were removed and homogenized. Normal females of the same age, anesthetized with 10 mg/100 g b.wt, were used as control animals. Microsomes were prepared as described by Philipp and Shapiro[6] by using a buffer containing 10 mM potassium phosphate, 2 mM EDTA, 1 mM dithiotreitol with or without 50 mM NaF. Electron microscope controls showed comparable morphology in the different preparations. Total cholesterol was assayed directly either on liver homogenate and microsomes, or on lipid chlorophorm-methanol extracts by using the Libermann-Buchard reagent (acetic-anhydride/sulphuric acid 19:1) and the same values were obtained. Proteins were estimated using the method of Lowry[7]. Total membrane phospholipid P_i was determined on a membrane chloroform-methanol extract according to the method of Morrison[8]; the phosphorus content was multiplied by 25 to calculate the amount of phospholipids. Phospholipid fractions of the extracts were separated as already reported[9]. Hepatic HMGCoA-reductase activity was tested according to Philipp and Shapiro[6] in 10 mM potassium phosphate, pH 7.2, 2 mM EDTA, 1 mM DTT, with or without 50 mM NaF. Microsomes were preincubated for 20 min at 37 °C. Cofactor mix was then added so that the final incubation medium contained: $5 \cdot 10^{-2}$ M glucose-6-P, $5.3 \cdot 10^{-3}$ M NADP, 1 unit of G-6P-dehydrogenase, $3.8 \cdot 10^{-2}$ M (1^{14}-C) mevalonic acid lactone (1132 dpm/nmole) and 5.10^{-4} M (^{3}H)-HMGCoA (4000 dpm/nmole). Incubation was carried out for 20 min at 37 °C.

Acetoacetyl-CoA-thiolase and HMGCoA-synthase activities were assayed in a 100,000 × g supernatant fraction using the spectrophotometric method[10].

(1-^{14}C)-acetate incorporation was tested in liver homogenates partially purified by 2000 × g centrifugation with an incubation medium containing: 0.1 M phosphate buffer, pH 7.4, 30 mM nicotinamide, 10 mM glutathione, 1 mM EDTA, 4 mM

Table 1. Rat liver protein content and cholesterol content in liver and in blood during pregnancy

	Control	Gestational age 16 days	19 days	22 days
Liver protein content (mg/100 g wet wt)	23.7 ± 8.1	19.0 ± 2.5	16.5 ± 3.2	25.3 ± 1.8
Liver cholesterol content (μg/mg protein)	21.5 ± 1.9	–	24.4 ± 2.1	29.2 ± 3.4
Blood cholesterol content (mg/100 ml plasma)	75.4 ± 4.1	80.2 ± 5.2	–	121.6 ± 6.3*

* $p < 0.001$ as determined by Student's t-test with respect to control animals; the data are the mean of 5 experiments ± SD.

Table 2. C^{14}Acetate incorporation in liver digitonin-precipitable sterols and HMGCoA reductase, HMGCoA synthase, AcetoacetylCoA thiolase activities during pregnancy

	Control	Gestational age 16 days	19 days	22 days
C^{14} Acetate incorporation (nmoles/h/g wet wt)	30.61 ± 2.55	30.99 ± 2.32	28.84 ± 2.12	1.15 ± 0.81*
HMGCoA reductase activity: (nmoles/min/mg protein)				
+ NaF	0.17 ± 0.01	0.12 ± 0.01	0.18 ± 0.01	0.04 ± 0.01*
− NaF	0.46 ± 0.05	0.38 ± 0.04	0.45 ± 0.03	0.13 ± 0.01*
HMGCoA synthase activity (nmoles/min/mg protein)	28.51 ± 1.95	16.91 ± 0.92*	16.74 ± 1.38*	2.32 ± 0.11*
AcetoacetylCoA thiolase activity (nmoles/min/mg protein)	75.43 ± 5.42	24.90 ± 1.10*	23.52 ± 1.73*	20.24 ± 1.83*

Data are the mean of 5 experiments; * $p < 0.001$ as determined by Student's t-test with respect to control animals.

Table 3. Cholesterol content, phospholipid content and percent composition of liver microsomes during pregnancy

	Control	Gestational age 16 days	19 days	22 days
Cholesterol content (μg/mg protein)	35.1 ± 2.4	33.6 ± 2.83	36.1 ± 2.2	38.3 ± 2.9
Phospholipid content (μg/mg protein)	605.3 ± 35	593.3 ± 3.2	635.4 ± 39	640.2 ± 37
% Phospholipid composition:				
sphyngomyelin	8.9 ± 0.9	10.9 ± 1.2	9.8 ± 1.1	9.1 ± 0.9
phosphatidylcholine	54.0 ± 2.2	48.0 ± 2.9	52.3 ± 4.5	53.2 ± 4.7
phosphatidylserine + phosphatidylinositol	15.4 ± 2.1	19.0 ± 2.3	15.2 ± 1.8	15.8 ± 1.2
phosphatidyl ethanolamine	23.1 ± 1.2	22.1 ± 1.3	22.4 ± 1.4	21.5 ± 0.9
Cholesterol/phospholipid ratio	0.058	0.056	0.057	0.059

Data are the mean of 5 experiments ± SD.

$MgCl_2$, 90 mM (1-^{14}C)-acetate sodium salt (specific activity 1270 dpm/nmole) for 30 min at 37 °C according to Gould and Swyryd[11]. At the end of incubation, samples were stopped with an equal volume of alcoholic 16% KOH, hydrolised for 90 min at 90 °C and extracted 3 times by means of 3 volumes of petrol ether. The radioactivity of digitonin-precipitable sterols was determined on the extracts.

Results and discussion. Liver protein and cholesterol content in the liver were constant with respect to the controls during pregnancy from days 16–22, while blood cholesterol content was higher at the end of pregnancy, has already been reported by several authors[12] (table 1). Acetate incorporation into digitonin-precipitable sterols was in agreement with controls at days 16 and 19 of pregnancy and fell markedly at the end of pregnancy just before parturition. Similar data have been reported for general lipogenesis by other authors[5,13]. The decreased rate of cholesterogenesis does not appear to be related to liver cholesterol content, which was normal. The same pattern was detected for HMGCoA-reductase activity of microsomes prepared either in the absence or in the presence of NaF. HMGCoA-synthase activity was lower than that in the control animals at days 16 and 19 and fell again at the end of pregnancy, while the acetoacetyl-CoA-thiolase was always low and constant during late pregnancy (table 2). Cholesterol content and phospholipid composition of microsomes appeared not to be changed with respect to the controls (table 3). Therefore, as in other systems[14], the cholesterogenesis pattern paralleled that of HMGCoA-reductase activity, while some other enzymes involved in HMGCoA synthesis showed different patterns. On the other hand, it is known that both synthase and acetylCoA-thiolase are enzymes correlated with metabolic pathways other than cholesterogenesis. The decreased reductase activity is not correlated with a different composition of microsome lipids; in fact, no variation during pregnancy was observed by us. The effects of pregnancy on cholesterol synthesis in the liver are probably hormonally induced. The decrease in liver cholesterogenesis is paralleled by a decrease in plasma insulin concentration[5]. Such a decrease of insulin might explain the inhibition observed in liver cholesterol synthesis, since insulin is clearly involved in the regulation of liver cholesterogenesis[15]. Other hormones may be involved in the regulation of cholesterogenesis during pregnancy; for instance it is known that HMGCoA-synthase activity as well as cholesterol synthesis are inhibited by cortisol in some cultured cells[16] and that the plasma cortisol content is much enhanced before parturition in some species. On the other hand, the enhanced levels of oestrogens – which are positive effectors of HMGCoA-reductase at physiological levels[17] – seem to have no effect on cholesterogenesis before parturition.

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