Hypolipidemic effect of pregnancy in the rabbit

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Abstract New Zealand white rabbits showed large decreases in plasma cholesterol and phospholipid concentrations during the second half of pregnancy. All lipoproteins (very low density, low density, and high density) participated in the decrease. Very large decreases in plasma cholesterol concentrations were observed even when the animals were maintained on high cholesterol diets. Increases in plasma cholesterol concentrations, after the intravenous administration of Triton WR 1339, were at least as great in pregnant as in nonpregnant animals. It is concluded that the decrease in plasma cholesterol concentrations is not the result of impaired plasma lipoprotein production.

Supplementary key words cholesterol · phospholipid · triglyceride · Triton WR 1339

Nearly fifty years ago Baumann and Holly (1) observed that in the pregnant rabbit, in contrast to several other species including man, the serum cholesterol decreased markedly during the latter part of pregnancy. This observation was extended by Popják (2), who showed that other lipids also decreased in the maternal blood plasma, and that even in rabbits fed cholesterol there was a hypolipidemic effect of pregnancy. Popják (2) rejected the hypothesis of Baumann and Holly (1) that the decreased serum lipids resulted from the utilization of maternal lipid by the growing fetus. Instead, he proposed that a hormonal or some other factor associated with pregnancy is responsible, possibly by shifting cholesterol from blood into the maternal tissues.

In the present paper we have reexamined this question and, in particular, the hypothesis that the decrease in maternal plasma lipids might be the result of a decreased secretion of lipids or lipoproteins by liver and other organs that normally maintain blood lipid concentrations at their steady state concentrations.

Abbreviations: VLDL, very low density lipoproteins.

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MATERIALS AND METHODS

The preparation of diets and management of the animals have been described in detail elsewhere (3). In brief, adult New Zealand white rabbits were utilized. All animals in this study were bred by artificial insemination. For a given experiment, semen was collected from a single buck by the method described by Bredderman, Foote, and Yassen (4). After establishing that the sperm were motile, the semen was diluted with 0.9% NaCl. Females were injected intravenously with 5 mg of pituitary luteinizing hormone (Armour-Baldwin Laboratories, Omaha, Neb.), and immediately thereafter 0.5 ml of the diluted semen was injected into the vagina (5).

The individually caged animals were fed Purina rabbit laboratory chow (Ralston Purina, St. Louis, Mo.) with or without the addition of cholesterol (250 mg) (Nutritional Biochemicals, Cleveland, Ohio) dissolved in 2.6 g of Wesson Oil (Hunt-Wesson Foods, Fullerton, Calif.). Daily food consumption records were kept and animals were weighed periodically. In some experiments, to assure consumption of the dietary cholesterol, this supplement was fed in a small amount of diet before the rest of the cholesterol-free diet was given. When this was not done, some of the pregnant animals that reduced their food consumption towards the end of pregnancy did not maintain a constant intake of cholesterol. In general, the inseminated animals were fed 100 g of Purina chow during the first 15 days after insemination and 150–200 g thereafter. Those animals kept with their litters during the first 10 days of lactation received 200 g of diet with or without added cholesterol.

Some rabbits were injected intravenously with Triton WR 1339 (200 mg/kg, Ruger Chemical Co., Irvington, N.Y.) in 0.9% NaCl. 16 hr later, and at various times thereafter, the animals were bled from the marginal ear vein. All blood samples were collected in tubes with 0.01 ml of 15% EDTA/ml of blood. Plasma cholesterol

was determined after saponification (6) and extraction with petroleum ether by the method of Zak et al. (7). Plasma triglyceride was determined by the modified method of Sardesai and Manning (8, 9), after separating the triglycerides from phospholipids on silicic acid columns (10). Phospholipids were determined by phosphorus analysis (11) of a purified lipid extract (12). Plasma lipoproteins were separated in a Spinco ultracentrifuge (40.3 rotor, 114,000 *g* avg) at 15°C (13).

RESULTS

In the first experiment, female rabbits fed Purina laboratory chow were bled repeatedly from 8 days before artificial insemination until days 25–28 of pregnancy. Fig. 1 shows the results obtained on five pregnant and one nonpregnant animals. The latter presumably ovulated in response to the injection of luteinizing hormone, but it is uncertain whether implantation took place since the animal was not killed. Plasma cholesterol concentrations in all pregnant animals decreased to very low levels towards the end of pregnancy. Most of this decrease occurred during the second half of pregnancy. One animal (1119) represented in Fig. 1 showed an abnormally high cholesterol increase in a single sample taken at the time of killing (day 26). This was the only animal that showed such an increase, but repeated analyses have shown that it is not an analytical mistake.

Fig. 2 shows data obtained from seven additional animals. One of them (1161) did not become pregnant after artificial insemination. All of the pregnant animals showed a pronounced minimum in their plasma cholesterol concentrations at the end of pregnancy with a sudden upswing after parturition and then a second decrease during early lactation. Animal 1161 (Fig. 2) presumably went through pseudopregnancy with more or less stable plasma cholesterol concentrations until day 33 after insemination. This occurred at the same time that the animals with young showed a sudden upturn in plasma cholesterol concentration. In order to exclude the possibility of a methodological error, frozen aliquots of plasma samples were reanalyzed with the same results.

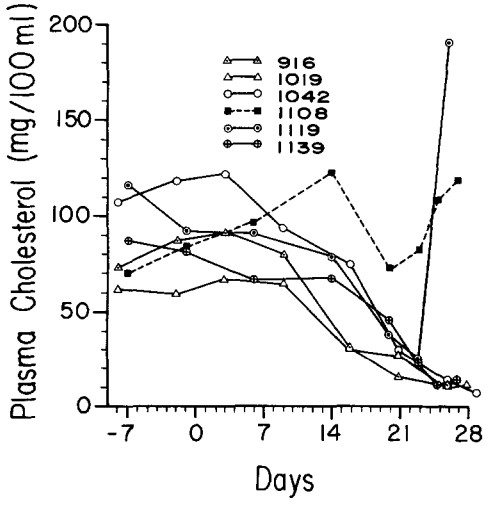

Fig. 1. Plasma cholesterol concentrations of pregnant rabbits maintained on a cholesterol-free diet. Animal 1108 (- - -) was inseminated but did not have any young. In this and in all subsequent figures, the day of insemination is shown as day 0 and code numbers refer to animal numbers.

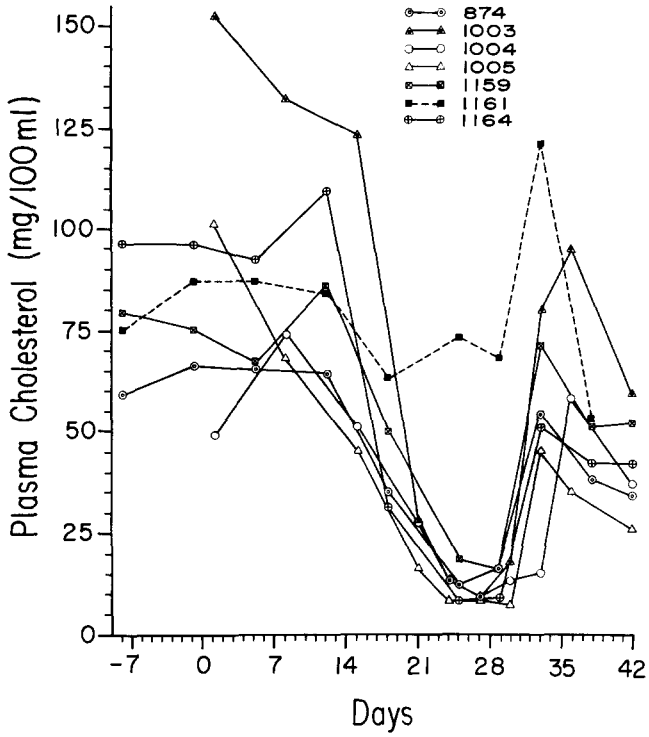

Fig. 2. Plasma cholesterol concentrations of pregnant and lactating rabbits maintained on a cholesterol-free diet. Animal 1161 (- - -) was inseminated but did not have any young. Animal 874 had young on day 31, animal 1004 on day 33, and the others on day 32.

The pronounced decreases in plasma cholesterol concentration occurring during pregnancy do not appear to depend on seasonal factors, since these studies have been repeated at various seasons for several years.

Plasma volumes, measured with Evans blue, appeared to be 40% larger in a group of 11 rabbits on day 28 of pregnancy than before insemination, but plasma protein concentrations were the same at both times.[1]

In Table 1, analyses of plasma lipids during pregnancy and early lactation are shown. The phospholipid concentrations appear to change nearly as much as cholesterol, but the triglyceride concentrations appear to be

[1] Ross, A. C., and D. B. Zilversmit. Unpublished observations.

TABLE 1. Plasma lipids in pregnant rabbits

Animal	Cholesterol Days[a] 1–15	Cholesterol Days[a] 21–27	Cholesterol Days[a] 33–42	Phospholipid Days[a] 1–15	Phospholipid Days[a] 21–27	Phospholipid Days[a] 33–42	Triglyceride Days[a] 1–15	Triglyceride Days[a] 21–27	Triglyceride Days[a] 33–42
				mg/100 ml					
1003	137	17	78	154	23	158	155	37	140
1004	58	16	37	101	28	73	71	56	68
1005	71	11	35	99	29	89	60	45	54

[a] Days after artificial insemination. Days 33–42 represent the period after parturition. Equal samples of plasma taken throughout the three periods were pooled for analysis.

somewhat more stable. However, the triglyceride values probably depend to a greater extent upon the time of the last feeding and may, therefore, be a less reliable index of endogenous lipid metabolism.

In Fig. 3 are shown the plasma cholesterol concentrations of seven pregnant and two inseminated but apparently nonpregnant rabbits fed 250 mg of cholesterol/day. The cholesterol regimen was begun 7 or 8 days before insemination of the rabbits. The two infertile animals showed a rapid increase in plasma cholesterol. In one of the animals (1112) the increase continued for the full 5 wk of the study, whereas the plasma cholesterol of the other one (1131) leveled off after 3 wk of cholesterol feeding. The latter animal's plasma cholesterol concentration showed a decrease at the same time as that of the pregnant animals. The fertile rabbits showed an increase in plasma cholesterol during the first 2 wk of pregnancy, followed by a sharp decline in serum cholesterol concentrations until the day of killing (day 26–30). The concentrations at that time were not as low as those in animals maintained on cholesterol-free diets, but the absolute decreases in plasma cholesterol concentrations from peak levels were much larger. In three animals this decrease amounted to between 800 and 900 mg/100 ml of plasma.

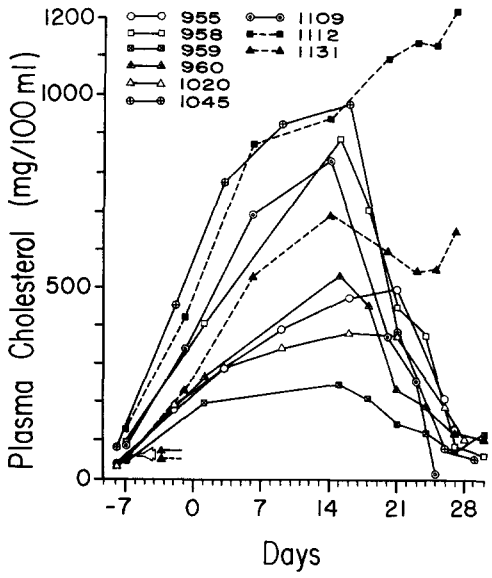

FIG. 3. Plasma cholesterol concentrations of pregnant cholesterol-fed rabbits. (——), pregnant animals; (- - -), inseminated but not pregnant, as determined for animal 1131 by surgical exploration on day 28. Animals 959, 960, 1020, 1109, 1131, and 1112 consumed 250 mg of cholesterol/day throughout; the remaining animals consumed 250 mg until day 21. Animal 958 consumed 200 mg/day after day 21, whereas animals 955 and 1045 decreased their food and cholesterol intake by variable amounts after days 22 and 23, respectively.

One must be cautious in assigning the entire decrease in plasma cholesterol to the effect of pregnancy. Although in most animals the dietary intake of cholesterol remained constant to the end of the experiment, some animals reduced their food and cholesterol consumption to variable extents. In animal 1045 (Fig. 3), for example, the level of plasma cholesterol decreased from 974 mg/100 ml to 388 mg/100 ml in 5 days, while dietary intake of cholesterol stayed at 250 mg/day. During the next 5 days the plasma cholesterol concentration decreased to 81 mg/100 ml, but the animal reduced its food intake drastically, eating about half as much cholesterol as before.

Plasma from three cholesterol-fed animals (958, 959, and 960) (Fig. 3) was centrifuged for 14 hr at 114,000 *g* at densities 1.006 and 1.063 in order to separate three lipoprotein fractions. Table 2 shows that at midpregnancy and near term the very low density and low density lipoprotein fractions contained most of the cholesterol. The percentage of plasma cholesterol in the very

TABLE 2. Lipoprotein cholesterol at days 15 and 30 of pregnancy

	Animal 958 Day 15	Animal 958 Day 30	Animal 960 Day 15	Animal 960 Day 30	Animal 959 Day 15	Animal 959 Day 30
Plasma total cholesterol (mg/100 ml)	884	65	528	118	248	102
$d < 1.006$ (%)[a]	70	67	54	53	39	41
$1.006 < d < 1.063$ (%)[a]	25	17	41	31	50	43
$d > 1.063$ (%)[a]	5.3	16	5.1	16	11	16

[a] Lipoprotein cholesterol is expressed as a percentage of total plasma cholesterol. Average recovery of cholesterol was 103%, but data have been normalized to 100% recovery.

low density lipoprotein fraction remained the same, whereas that in the low density fraction appeared to be relatively lower near the end of pregnancy. Although as a percentage of the total cholesterol the high density fraction (d > 1.063) appears to increase later in pregnancy, the absolute amount of cholesterol in this fraction as well as in the other fractions is less at that time than at midpregnancy. Thus, it appears that all three lipoprotein fractions decrease during pregnancy.

In animals on cholesterol-free diets (Fig. 2), the plasma cholesterol concentrations appeared to increase rapidly after parturition but the concentrations remained suppressed during early lactation. The same phenomenon was observed in rabbits maintained on an intake of 250 mg of cholesterol/day (Fig. 4). In two animals, plasma cholesterol concentrations decreased to 23 and 33 mg/100 ml and then rose to 89 and 223 mg/100 ml during early lactation. It appears that in cholesterol-fed animals as well as in the animals on cholesterol-free diets the plasma cholesterol concentration is suppressed during early lactation.

In the next set of experiments the influx of cholesterol and triglyceride into blood plasma was measured by administration of 200 mg of Triton WR 1339/kg of body weight. Inasmuch as we did not wish to subject the pregnant animals to prolonged fasting, we determined the difference in blood lipid response between the fasted and fed states in a preliminary experiment. In Table 3 are compared the increases of plasma cholesterol and triglycerides in normally fed rabbits and in animals fasted 24 hr before Triton injection. The influx of plasma cholesterol, as measured by this technique, was the same in the fed and fasted states. This is not unexpected since the diet contained no cholesterol. Triglyceride influx was quite variable and appeared to be somewhat higher in the fed state.

TABLE 3. Increases in plasma cholesterol and triglyceride in fed and fasted rabbits 16 hr after Triton administration

Animal[a]	Plasma Cholesterol		Plasma Triglyceride	
	Fed	Fasted	Fed	Fasted
	mg/100 ml		*mg/100 ml*	
955	116	122	1790	1001
1036	127	106	1872	774
1042	140	121	2281	1599
1045	110	145	1158	1426
Avg ± SE	123 ± 6.6	123 ± 8.0	1775 ± 232	1200 ± 190

[a] Triton was first injected into normally fed animals. 8 wk later the animals received their last meal about 24 hr before Triton injection and food cups were removed 20 hr before Triton injection.

In Fig. 5 are shown the alterations in plasma cholesterol concentrations in four rabbits fed a cholesterol-free diet and given Triton WR 1339 2 wk before insemination and on day 20 of pregnancy. The plasma cholesterol response to Triton appears to be linear for about 2.5 days and to be similar in the pregnant and nonpregnant states. The plasma cholesterol increments due to Triton are summarized for these four animals plus an additional animal in the top portion of Table 4.

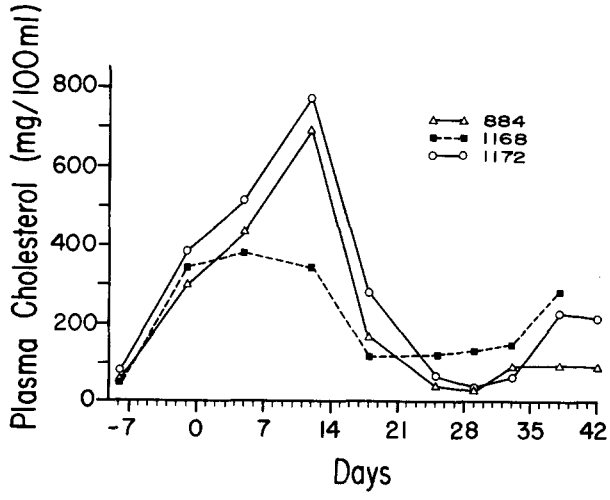

FIG. 4. Plasma cholesterol concentrations of pregnant and lactating cholesterol-fed rabbits. Animals 1172 and 884 had three and five young, respectively, on day 32. Animal 1168 did not have any young; yet the pronounced decrease in plasma cholesterol after day 12 suggests that this animal was pregnant and possibly aborted. All animals ate 250 mg of cholesterol/day except on days 29 and 30 when animal 1172 ate only little food and 30 mg of cholesterol.

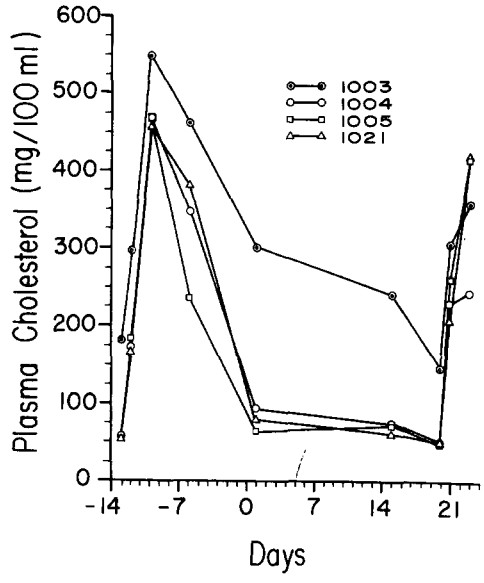

FIG. 5. Effect of Triton WR 1339 on plasma cholesterol. Triton was administered intravenously (200 mg/kg) 14 days before insemination, which was on day 0, and on day 20 of pregnancy. Animals were killed on day 25, all showing evidence of pregnancy. Animals 1003, 1004, and 1005 each had six or seven live fetuses. Animal 1021 had no live fetuses at the time of surgery, but a dead fetus was found in the cage, at least two implantation sites were present in the left horn, and 12 corpora lutea were present in the two ovaries.

TABLE 4. Plasma cholesterol before and after Triton WR 1339 administration

Nonpregnant					Pregnant				
Animal	Wt	Before Triton	16 hr after Triton	Increase	Animal	Wt	Before Triton	16 hr after Triton	Increase
	kg		*mg/100 ml*			*kg*		*mg/100 ml*	
957	4.24	50	198	148	957	4.61	11	58	47
1003	4.16	179	294	115	1003	4.55	143	303	160
1004	4.60	58	171	113	1004	4.95	50	227	177
1005	4.00	52	183	131	1005	4.30	47	257	210
1021	4.78	53	166	113	1021	4.99	49	206	157
955	5.21	37	153	116	971	4.58	37	245	208
1050	4.99	68	275	207	1102	4.54	23	177	154
1052	3.69	51	133	82	1122	4.40	25	168	143
1099	4.65	64	219	155	1136	4.77	46	225	179
1101	4.60	60	157	97	1170	4.27	30	218	188
1175	4.15	53	165	112					
Avg increase ± SE				126 ± 10					162 ± 15

Top portion of table shows plasma cholesterol response to Triton WR 1339 in the same animals before pregnancy (left) and at day 20 of gestation (right). The bottom portion shows the response to Triton in six nonpregnant animals (left) and in five animals on day 20 of gestation (right).

In these animals, abortions or resorbed fetuses were noted when the animals were explored surgically on day 25. Because the possibility existed that two consecutive doses of Triton had interfered with the normal development of the fetus, we did an additional experiment in which the response to Triton in five pregnant rabbits was compared with that in six nonpregnant controls. When the pregnant animals had reached day 20 of gestation, all 11 animals were fed at 8 AM and food was removed at 10 AM. At 5 PM of that day, "zero-time" blood samples were taken and the animals were injected with Triton WR 1339. Food was withheld for the next 16 hr, at which time the animals were bled again and killed. The plasma cholesterol data are shown in the bottom portion of Table 4. The average increase in plasma cholesterol for all animals treated with Triton WR 1339 was 162 mg/100 ml in the pregnant rabbits and 126 mg/100 ml in the controls. These data indicate that the secretion of cholesterol into plasma of the pregnant animals is not decreased below normal levels. On the contrary, the data suggest that cholesterol secretion in the pregnant animals may be somewhat higher in the controls.[2]

DISCUSSION

Alterations of plasma lipid levels during pregnancy vary in different species. Green (14) reported that human serum cholesterol increased throughout pregnancy and was maximal at or near term. Baumann and Holly (1) and Popják (2) reported that in rabbits, serum cholesterol and phospholipids decreased during pregnancy, whereas in dogs, little or no change in serum lipids took place. More recently, Ho, Forestner, and Manalo-Estrella (15) observed very low serum cholesterol concentrations in rabbits at term. The difference between rabbits and women cannot be explained on the basis of the vegetarian diet of the former, since Mullick, Bagga, and Du Mullick (16) observed that in pregnant vegetarian women serum cholesterol increased.

Decreases in plasma cholesterol of the pregnant monkey (*Macaca mulatta*) have been reported by Wolf, Temte, and Meyer (17) and by Martin, Wolf, and Meyer (18). In the latter study, both phospholipids and cholesterol of plasma reached a minimum level at about midpregnancy and showed a definite rise before parturition. In the pregnant baboon, smaller decreases in serum cholesterol have been observed (19).

The rapid decrease of plasma cholesterol in the pregnant rabbit offers a good opportunity for the investigation of control mechanisms. In principle, the decrease in plasma cholesterol could result from: (*1*) hemodilution, (*2*) decreased secretion of plasma lipoprotein by liver and other organs that normally produce them, (*3*) transfer of maternal cholesterol to the fetuses, (*4*) increased utilization of cholesterol for steroid hormone biosynthesis, (*5*) increased excretion of sterols and bile acids in the feces, (*6*) storage of cholesterol in maternal tissues, and (*7*) in the case of the cholesterol-fed animal, a decrease in absorption of dietary cholesterol.

In the present investigation we have described the changes in plasma cholesterol at various stages of pregnancy and during early lactation in animals fed diets with and without added cholesterol. In addition, we have studied lipoprotein secretion into plasma by the use of Triton WR 1339. The results show that in animals on

[2] The *t* value for the difference between means calculated as two independent samples was 2.05, whereas a value of 2.09 would indicate a significance at the 5% level.

a cholesterol-free diet, plasma cholesterol concentrations of the pregnant rabbit remain relatively constant during the first half of pregnancy. After that time they decrease sharply, and in some animals reach levels of 5 mg/100 ml. In all animals the lowest cholesterol concentrations were reached between day 25 and the time of parturition (day 31–33). Sharp increases in serum cholesterol were observed within 1 or 2 days after parturition. This increase was followed by a second but smaller decrease during early lactation.

The decrease of plasma cholesterol in late pregnancy might have resulted in part from hemodilution, although hematocrits and plasma protein concentrations were not significantly altered during pregnancy.

The decrease in plasma cholesterol concentrations during the second half of pregnancy was not prevented by the addition of cholesterol to the diet, but in absolute terms was actually exaggerated. Food consumption of some animals maintained on high cholesterol diets decreased during the latter part of pregnancy, but even in those animals in which cholesterol and food intake was constant throughout pregnancy the plasma cholesterol values decreased to very low levels.

The rate at which triglyceride is secreted into the bloodstream has been determined by measuring the accumulation of triglyceride in the plasma after the administration of Triton WR 1339, a detergent that blocks the clearance of plasma triglycerides. This method has been tested widely in rats and has been found to give results comparable to radiotracer procedures (20). The quantitative aspects of the Triton procedure have not been tested for the measurement of cholesterol secretion rates. However, there is good evidence that cholesterol is secreted into the blood plasma as part of the very low density lipoprotein complex and that the clearance of cholesterol is associated with the removal of triglyceride from this complex (21).

Yamada et al. (22) tested the action of Triton WR 1339 on plasma cholesterol in the rabbit and found that a dose of 200 mg/kg produced a nearly linear increase in plasma cholesterol for 24 hr. In our animals the same dose of Triton produced a linear increase in plasma cholesterol concentrations for about 2.5 days. The rate of plasma cholesterol increase in our control rabbits of 7.9 mg/100 ml/hr agrees reasonably well with the 11.3 mg/100 ml/hr reported by Yamada et al. (22). In our studies the increase in plasma cholesterol was accompanied by an increase in plasma triglycerides which, in the fasting animals, was 10 times as high as that for cholesterol. Since the increment of plasma cholesterol and triglyceride is due primarily to the accumulation of very low density lipoproteins, it is of interest that this ratio of 10 is considerably higher than the triglyceride-to-cholesterol ratio of 5.9 reported for circulating VLDL in rabbit plasma (23). However, such a difference would be expected if the VLDL in the plasma of Tritonized animals represents newly secreted VLDL whereas circulating VLDL of normal rabbits represents VLDL from which part of the triglyceride has been removed by peripheral lipolysis. Indeed, the triglyceride-to-cholesterol ratio of VLDL isolated from the Golgi apparatus of rat liver is reported to be 7 (24), whereas that of circulating VLDL in rats fasted for 3.5 hr is 4 (24) and for those fasted 16 hr is 2 (25).

The rate at which cholesterol was secreted into the plasma of the pregnant rabbits was 10.1 ± 1 mg/100 ml/hr. This value, instead of being lower, is actually somewhat higher than the 7.9 ± 0.6 found for the nonpregnant animals, the difference being just below the 5% significance level. It can be concluded, therefore, that the decrease in plasma cholesterol during pregnancy does not result from an impairment in the secretion of plasma cholesterol.

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