Cholesterol Metabolism and Placental Transfer in the Pregnant Rhesus Monkey

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ABSTRACT The placental transfer of cholesterol (5-cholesten-3β-ol) was investigated by giving pregnant rhesus monkeys cholesterol-1α-^{3}H or cholesterol-4-^{14}C and then determining the cholesterol specific activity (SA) in maternal serum and in fetal serum and tissues. An isotopic steady state was established in five pregnant animals by the daily feeding of a tracer dose of cholesterol-4-^{14}C. Comparison of maternal and fetal serum cholesterol SA revealed that an average of 42.6% of the serum cholesterol in the term fetus originated by transfer from the maternal blood. The remainder presumably arose by fetal synthesis *de novo*. Fetal tissues had cholesterol SA equal to or slightly less than that of fetal serum, except for brain which had a SA only 5% that of fetal serum.

In other studies a single intravenous dose of radioactive cholesterol was given to either mother or fetus in late pregnancy. The time for detectable passage across the placenta in either direction was between 4 and 24 hr. With maternal administration of the isotope, there was equilibration of maternal and fetal serum cholesterol SA after 10–12 days. With fetal injection of isotopic cholesterol, however, the maternal cholesterol SA never attained a level more than 5% of fetal SA. This indicated that the net cholesterol flux was strongly in the direction of mother to fetus.

Serum cholesterol levels were significantly greater in maternal than in fetal serum (80.3±18.5 vs. 59.6±15.6 mg/100 ml). Maternal serum cholesterol concentration in the monkey was significantly lower in late pregnancy than during the puerperium. Studies of breast milk indicated that approximately two-thirds of milk cholesterol was transferred from the maternal blood.

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INTRODUCTION

The origin of fetal cholesterol—whether transferred from the maternal blood or synthesized *de novo* by the fetus—has long been a matter of interest. Experimental studies of this problem have produced seemingly contradictory results. Early studies in the rat indicated a limited degree of transfer of deuterium-labeled cholesterol from mother to fetus (1). Popjak and Beeckmans (2), on the other hand, found no evidence of placental transfer of radioactive cholesterol in the rabbit. They concluded that fetal cholesterol originated entirely by fetal synthesis. Chevallier (3) produced an isotopic steady state in pregnant rats by feeding cholesterol-4-^{14}C and found that 60–70% of the cholesterol in the 12 day fetus was of maternal origin while only 10–15% of the cholesterol in the term fetus originated from the mother. Connor and Lin (4) determined that 22% of the fetal cholesterol in the guinea pig was of maternal origin and further found evidence of maternal-fetal transfer of cholesterol within 2 days in the pregnant rabbit.

The present report concerns placental transmission of isotopic cholesterol in a primate, the rhesus monkey (*Macaca mulatta*). The placenta of the rhesus macaque is hemochorial in type and is both structurally and functionally similar to that of the human in that the maternal and fetal circulations are separated by only two cell layers. In addition to placental transfer, the concentration of radioactive cholesterol in various fetal tissues was measured, permitting certain conclusions about the origin of tissue cholesterol. Other aspects of cholesterol metabolism during pregnancy and the puerperium, including the excretion of cholesterol into breast milk, were studied.

METHODS

Two types of investigations were done—steady-state studies in which an isotopic steady state was established in the pregnant rhesus monkey by the feeding of radioactive cho-

lesterol in the daily diet and single dose studies in which an intravenous injection of the labeled cholesterol was given to either mother or fetus. The labeled forms of cholesterol used were cholesterol-4-^{14}C (SA 55.2 mCi/mmole, New England Nuclear Corp., Boston, Mass.) and cholesterol-1α-^{3}H (SA 10.4 Ci/mmole, Amersham/Searle Corp., Arlington Heights, Ill.). Before use, the purity of the isotopes was verified by thin-layer chromatography (TLC) as described by Mangold (5).

Steady-state studies. Five pregnant monkeys, estimated to be in the midportion of pregnancy, were fed a daily dose of 2 μCi cholesterol-4-^{14}C to achieve an isotopic steady state. The daily ration consisted of 100 g of Purina monkey chow (Ralston Purina Co., St. Louis, Mo.) which has a low cholesterol content (9.6 mg cholesterol/100 g analyzed by us) and contains approximately 5% fat. An ether solution containing 2 μCi cholesterol-4-^{14}C, 1 mg nonradioactive cholesterol, and 2.5 g peanut oil was mixed with 100 g of this chow and the ether solvent then evaporated. Even distribution of the isotope in chow prepared this way was verified by determining radioactivity of small portions of the chow selected at random. At weekly intervals, fasting blood samples were drawn from a maternal peripheral vein and the concentration and SA of serum cholesterol were determined. The weights of the monkeys were measured weekly. Their mean body weights increased from 6.15 kg 5 wk before parturition to 6.46 kg at parturition. The mean weight decreased from 5.42 kg the 1st wk after birth to 4.83 kg 5 wk later.

Three of the animals were delivered by hysterotomy. The other two animals gave birth spontaneously before scheduled abdominal delivery and the young were separated from their mothers within eight hr of birth. All newborns were healthy and appeared at term. Their weights were 336–516 g. Blood specimens were obtained from the newborns by cardiac puncture; they were later killed by intracardiac pentobarbital. A variety of fetal tissues was taken for analysis. The intestinal contents were washed from the mucosa with saline. The intestinal segments were divided into mucosal and muscular wall components for separate analyses by scraping off the mucosa with a spatula. Milk was expressed from the breasts of each of the mothers on the 2nd day after delivery. Maternal blood collection was continued at weekly intervals into the puerperium.

Single dose studies. Cholesterol transfer from mother to fetus after maternal intravenous injection was investigated in a total of 11 animals. In two of these monkeys (A and B), the early maternal-fetal transfer of cholesterol-4-^{14}C was studied during the 1st 4 hr after administration in pregnant animals near term (fetal weights subsequently found to be 368 and 402 g). For these studies, the pregnant monkeys were premedicated with phencyclidine hydrochloride (Sernylan, Parke, Davis & Co., Detroit, Mich.) 2 mg/kg and atropine 0.1 mg intramuscularly, and anesthetized with ethyl ether. The abdomen was then opened and the uterus was transilluminated for identification of interplacental vessels (6). An interplacental vein was catheterized with a silastic T tube (Extracorporeal Medical Specialties, Inc., King of Prussia, Pa.). This then permitted sequential sampling of fetal venous blood with the fetus *in utero* and the amniotic sac intact. After injection of a sterile solution of 10 or 15 μCi cholesterol-4-^{14}C in 0.5 ml ethanol and 2.5 ml normal saline into a maternal peripheral vein, samples of maternal (from a catheter in the maternal vena cava) and fetal blood were obtained at 30-min intervals for 3 or 4 hr. At the end of this time, the fetus was delivered by hysterotomy. In each instance, the newborn was vigorous and breathed spontaneously within 30 sec.

Cholesterol transfer from mother to fetus over periods of time ranging from 1 to 26 days was investigated in nine other monkeys (C through K) estimated to be in late pregnancy. In these experiments, cholesterol-1α-^{3}H (40 μCi) was injected into a maternal saphenous vein. Maternal blood was collected at intervals of 1–3 days until delivery and cholesterol concentration and SA were determined.

In four of these nine animals, laparotomy was performed 5 or 7 days after maternal injection of cholesterol-1α-^{3}H. An interplacental vein was identified, isolated, and punctured with a 25 gauge needle. Fetal venous blood was withdrawn for determination of cholesterol content and SA. Cholesterol-4-^{14}C (10 μCi) was then injected into the fetal circulation. By applying pressure to the puncture site for approximately 5 min, bleeding was controlled without ligating the vessel. The uterine and abdominal incisions were then closed and pregnancy was permitted to continue. Maternal blood samples were drawn from a peripheral vein 1 and 4 hr after fetal isotopic injection and at 1- to 3-day intervals thereafter until delivery 3–19 days later. The SA of both cholesterol isotopes was determined.

Two of these nine monkeys (G and H) delivered vaginally 8 and 11 days, respectively, after maternal administration of cholesterol-1α-^{3}H; the other seven were delivered by hysterotomy. Fetal blood was collected by cardiac puncture in all newborn monkeys.

Sample analysis. In all experiments, cholesterol was extracted from serum, milk, and tissues and its concentration and SA were determined. The cholesterol concentration of serum and milk was measured by the method of Abell, Levy, Brodie, and Kendall (7). For the determination of radioactivity, the serum and milk were saponified with potassium hydroxide and the nonsaponifiable residues extracted with hexane, dried and dissolved in 10 ml scintillation mixture (2,5-diphenyloxazoyle [PPO] and 1,4-bis[2-(5-phenyloxazolyl)]benzene [POPOP] in toluene, Packard Instrument Co., Downers Grove, Ill.). Fetal tissues were dried under vacuum at 100°C, ground to a powder, and extracted with chloroform-methanol (8). The cholesterol concentration and SA of fetal tissue extracts were then determined by the same method used for analysis of serum and milk.

All specimens were counted in a Packard Tri-Carb liquid scintillation counter (Packard Instrument Co.) with absolute activity analyzer. In experiments in which both isotopic

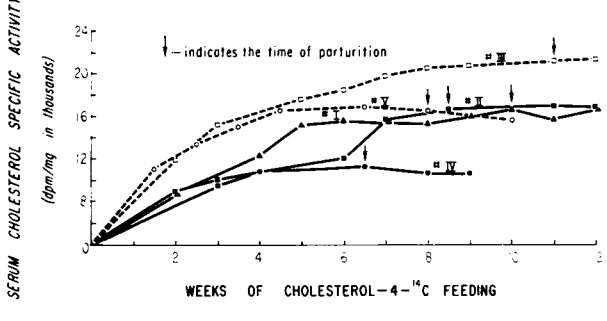

FIGURE 1 SA of maternal serum cholesterol in five pregnant monkeys fed cholesterol-4-^{14}C (2 μCi/day). An isotopic steady state was established 4–7 wk after feeding began.

TABLE I

Cholesterol Concentration and Radioactivity in Maternal and Fetal Sera under Steady-State Conditions

Monkey No.	Serum cholesterol		SA		Fetal serum SA as per cent of maternal serum SA
	Maternal	Fetal	Maternal	Fetal	
	mg/100 ml		*dpm/mg cholesterol*		
I	85	54	16,612	7,667	46.2
II	52	50	16,679	6,224	37.3
III	90	68	21,167	10,015	47.3
IV	104	47	11,337	5,085	44.9
V	93	66	16,409	6,091	37.1
Mean	85	57	16,441	7,016	42.6

forms of cholesterol were used in the same animal, the use of the dual label setting permitted differential counting of ^{14}C and ^{3}H activity.

That radioactivity in serum and liver extracts was confined to the cholesterol molecule was verified by TLC. Lipids were chromatographed on a silica gel plate (solvent system hexane : chloroform : ethyl ether : acetic acid 80 : 10 : 10 : 1) for fractionation into phospholipids, cholesterol, triglycerides, and cholesteryl esters. The band of each lipid was eluted with chloroform and its radioactivity determined. More than 95% of the radioactivity in fetal sera and liver examined in this manner was contained in the cholesterol and cholesteryl ester bands. That the sterols in the cholesterol and cholesterol ester bands were actually cholesterol (5-cholesten-3β-ol) was established as follows: eluents of the TLC plates were hydrolyzed to free form by saponification, converted to the trimethylsilyl ether derivatives of the sterol, and subjected to gas-liquid chromatography; single peaks with the same retention time as that of pure cholesterol standard were found.

Statistical analyses were carried out by the *t* and the "sign" tests (9).

RESULTS

Steady-state studies. The five pregnant monkeys fed a constant amount of cholesterol-4-^{14}C in the daily diet reached an isotopic steady state 4–7 wk after isotope feeding was begun (Fig. 1). Table I depicts the levels and specific activities of maternal and fetal serum cholesterol in these five animals at the time of delivery. Both absolute concentration and SA of cholesterol were higher in maternal than in fetal serum. The mean value of fetal serum SA expressed as a proportion of maternal serum SA was 42.6% (range 37.1–47.3%).

With the exception of brain all fetal tissues had cholesterol SA at least two-thirds that of fetal serum (Table II). As expected, since blood and liver are both components of the same cholesterol pool, the liver cholesterol SA was identical with that of serum. Heart muscle SA was also high but the cholesterol SA of skeletal muscle was lower (67.8% of serum SA). The intestinal specific activities ranged from 57.4 to 77.9% of serum and were similar for both the mucosa and the muscular wall components. The specific activities of ileal and colon contents ranged from 33.1 to 56.1% of fetal serum. Of three fetuses whose jejunal contents were examined, two contained no detectable cholesterol while the third had SA approximately the same as serum. Amniotic fluid, obtained from two monkeys, had the same specific activities as fetal serum. The fetal brain had low but de-

TABLE II

Cholesterol Radioactivity (dpm/mg) of Serum and Tissues

No.	Serum	Liver	Heart muscle	Skeletal muscle	Lung	Kidney	Stomach	Jejunum	
								Content	Mucosa
I	7,667	6,987	7,624	4,775	6,594	7,062	5,247	—	—
II	6,224	6,171	5,668	3,883	4,848	5,530	3,388	—	4,646
III	10,015	10,313	9,660	7,324	8,661	8,049	7,366	10,475	8,153
IV	5,085	4,595	4,662	3,540	4,121	4,356	2,713	—	3,765
V	6,091	6,194	5,393	4,267	4,716	5,784	4,386	—	—
Mean	7,016	6,851	6,601	4,757	5,788	6,156	4,620		5,521
Per cent of fetal serum SA		97.6	94.1	67.8	82.5	87.7	65.8		77.8

tectable radioactivity. Brain SA was only 4.6% that of serum.

The cholesterol content of brain, on the other hand, was four to five times that of other tissues (Table III). Some other tissues had higher cholesterol concentrations than adult monkeys (10), skeletal muscle 5.8 mg (fetus) vs. 1.8 mg (adult)/g of tissue; heart muscle 8.4 vs. 3.3 mg; liver 12.1 mg vs. 8.5 mg. Amniotic fluid had a low cholesterol concentration (1.6 and 7.5 mg/100 ml).

Single dose studies. Maternal-fetal transfer. In the 11 monkeys in which the radioactive cholesterol was administered to the mother, no radioactivity was detected in the fetal blood during the subsequent 4 hr (monkeys A and B, Table IV) despite high concentrations of radioactivity in the maternal circulation. However, evidence for cholesterol transfer into the fetal circulation was clearly demonstrated by 24 hr, fetal serum cholesterol SA reaching 1097 and 1333. By 7–9 days the ratio of fetal to maternal cholesterol SA had reached 0.55 (mean of five studies) and by 14–17 days the ratio exceeded unity. The final 26 day ratio was also above unity, 1.16 (SA). These data confirmed that cholesterol transfer into the fetus occurred, but gradually, and further suggested a precursor-product realtionship in which the transfer in the opposite direction (i.e., fetus to mother) did not occur to the same extent. The SA ratios above unity at a time when the expected exponential decay of maternal cholesterol SA was occurring indicated the possibility of relatively poor exchange.

Fetal-maternal transfer. Four fetuses were given isotopic cholesterol intravenously and the possible transfer across the placenta into the maternal circulation then studied (Table V). During the 1st 4 hr after fetal administration, no radioactivity was detected in the maternal blood in two animals and low levels in the other two animals. By 24 hr after fetal injection, all mothers had significant amounts of radioactive cholesterol in their sera. Subsequently, maternal levels increased further for 1–2 days, then appeared to decline. In all instances, however, the ratios of maternal to fetal cholesterol SA were very low, ranging from 0.019 (monkey G) to 0.045 in monkey K at 19 days after the fetal injection of the isotope. It is apparent that only trace amounts of cholesterol moved across the placenta from fetus to mother.

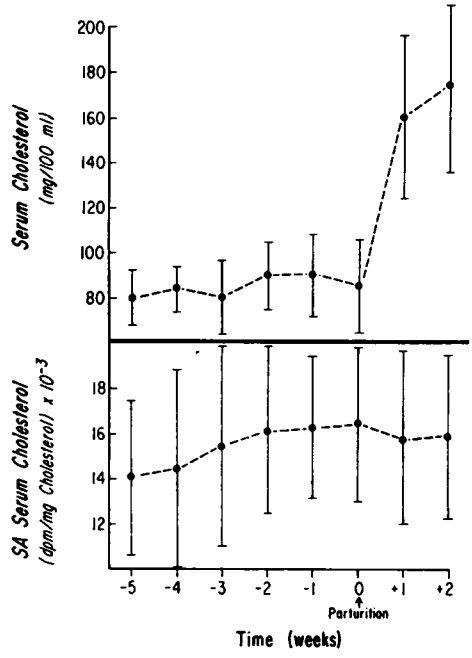

FIGURE 2 Mean (±SD) serum cholesterol values and SA of five monkeys during pregnancy and during the puerperium. The mean serum SA did not change although the serum cholesterol doubled in the puerperium.

Maternal-fetal serum cholesterol levels. For all 16 animals of the study, the mean maternal serum cholesterol concentration at delivery was 80.3 (±18.5 SD) mg/100 ml while the mean fetal serum cholesterol concentration was

of the Newborn Monkey under Steady-State Conditions

Jejunum	Ileum			Colon			
Muscular wall	Content	Mucosa	Muscular wall	Content	Mucosa	Muscular wall	Brain
5,240	—	—	5,486	—	—	3,752	371
4,858	3,211	4,026	4,066	1,687	2,484	3,417	280
8,500	6,340	7,841	8,122	3,850	4,754	5,966	471
3,457	2,409	3,589	3,349	1,525	2,408	2,693	202
5,279	—	—	4,993	—	—	4,303	303
5,467	3,987	5,152	5,203	2,354	3,154	4,026	325
77.9	56.1	72.5	74.2	33.1	44.4	57.4	4.6

TABLE III
The Tissue Cholesterol Content of the*

Monkey No.	Liver	Heart muscle	Skeletal muscle	Lung	Kidney	Stomach	Jejunum	
							Content	Mucosa
I	9.4	7.3	6.9	14.1	14.3	10.3	—	—
II	9.4	7.9	4.1	14.4	13.7	11.4	0	8.7
III	10.5	8.1	5.4	18.7	14.0	11.8	1.6	11.0
IV	15.9	9.5	7.0	14.3	18.8	15.4	0	6.9
V	15.1	9.4	5.7	17.1	17.4	12.4	—	—
Mean	12.1	8.4	5.8	15.7	15.6	12.3	0.5	8.9

* Cholesterol content of fetal tissues expressed as milligrams per gram dried weight.

59.6 (±15.6 SD) mg/100 ml. Maternal serum cholesterol level was significantly higher than the fetal level ($P < 0.01$).

Cholesterol concentrations and SA of maternal serum during pregnancy and during the puerperium presented marked contrasts (Fig. 2) in the five isotopic steady-state monkeys followed sequentially for long periods of time. These monkeys received the same diet and same amount of isotope postpartum as antepartum. The serum cholesterol levels doubled after delivery, 84–166 mg/100 mg. Surprisingly, the serum cholesterol SA did not decrease correspondingly but remained the same in the puerperium (Table VI).

Transfer of cholesterol into breast milk. Breast milk obtained on the 2nd day after delivery in the five steady-state animals contained cholesterol in amounts ranging from 19 to 51 mg/100 ml (Table VII). The SA of breast milk cholesterol varied from 58.7 to 76.8% of SA of maternal serum cholesterol. This indicated that approximately two-thirds of cholesterol in breast milk was transferred from the maternal blood since the secretion of milk involves a net production or transfer and not exchange (i.e., a precursor-product relationship).

Since two of the monkeys (I and IV) gave birth spontaneously during the night, it was conceivable that these newborns could have suckled radioactive milk and thereby have changed their serum SA. For the investigation of this possibility, the stomach contents of these newborns were analyzed and found to have cholesterol specific activities values of 11 and 59% of neonatal serum. These values were similar to those of the stomach contents of the three monkeys delivered by hysterotomy and examined immediately after birth. Moreover, the serum cholesterol specific activities of the animals born vaginally did not differ appreciably from those delivered abdominally. Therefore, we concluded that any possible ingestion of milk containing radioactive cholesterol had little effect on the serum values obtained.

TABLE IV
SA Serum Cholesterol (dpm/mg) in Mother (M) and Fetus (F)

Time after maternal injection*	Monkey											
	A		B		C		D		E		F	
	M	F	M	F	M	F	M	F	M	F	M	F
4 hr	2046	0	1673	0								
24 hr					6293	1097	2250	1333				
2–3 days									3286	615	1989	
4–5 days											1564	
7–9 days											1234	539
10–12 days												
14–17 days												
20–24 days												
26 days												

* Monkey A received 15 μCi cholesterol-4-^{14}C, monkey B received 10 μCi cholesterol-4-^{14}C, and all other monkeys received 40 μCi cholesterol-1α-^{3}H.

Newborn Monkey under Steady-State Conditions

Jejunum	Ileum			Colon			
Muscular wall	Content	Mucosa	Muscular wall	Content	Mucosa	Muscular wall	Brain
13.5	—	—	13.2	—	—	16.6	59.0
12.1	5.1	10.8	14.0	66.0	8.9	12.1	51.3
13.2	4.5	12.6	14.8	58.0	11.3	16.1	59.0
15.2	3.3	7.3	14.0	51.4	9.2	15.9	54.2
15.5	—	—	15.6	—	—	15.5	62.6
13.9	4.3	10.2	14.3	58.3	9.8	15.2	57.2

DISCUSSION

The experiments described in the pregnant rhesus monkey demonstrate conclusively that there was a net transfer of cholesterol from the maternal circulation into the fetus. The mere finding of isotopic cholesterol in the fetus could indicate only an exchange of cholesterol molecules between mother and fetus and not necessarily a net accumulation of cholesterol from the mother into the fetus. Net transfer was proven by experiments in which labeled cholesterol was given either to mother or fetus and the specific radioactivities of maternal and fetal blood then compared. Flux of cholesterol into the fetus occurred readily. Even in these experiments the period of time required for equilibration of fetal isotopic cholesterol with that of the mother suggested that the fetus as a whole was a component of a more slowly turning over pool, similar to pool B as described by Goodman and Noble (11).

This eventual equilibration from *mother to fetus* was not matched by comparable equilibration from *fetus to mother*. The amount of feto-maternal transfer was very slow. Even when 19 days after fetal administration was allowed for equilibration, the maternal SA remained less than 5% of the fetal SA. This indicated a positive gradient of cholesterol flux from mother to fetus in the pregnant primate. An even stronger positive gradient has been previously demonstrated in the chick embryo which quantitatively derives *all* of its cholesterol from the yolk of the egg (12). Even considering the small-cholesterol pool size of the fetus vs. the larger pool size of the mother (roughly 1 to 15), the dilution of fetal cholesterol transferred to the mother could not account for the very low maternal cholesterol SA (e.g., in monkey J, 598 compared with 17,542 in the fetus) unless the rate of transfer was extremely slow and incomplete.

In the experiments involving an isotopic steady state in pregnant monkeys, it is possible to make quantitative

after Maternal Administration of Radioactive Cholesterol

Monkey										
G		H		I		J		K		Mean ratio fetal to maternal serum SA
M	F	M	F	M	F	M	F	M	F	
										0
										0.38
2083		1828		5356		1303		2090		0.19
2378	692			3193		1010		1876		0.34
1208	650	1277	653	2494		778	392	1176	898	0.55
		926	516	1886		608		1010		0.56
				1114	1646	412	408	752		1.23
								539		
								422	490	1.16

TABLE V

SA Serum Cholesterol (dpm/mg) in Mother and Fetus after Fetal Administration of Radioactive Cholesterol

Monkey		Time after fetal injection of 10 μCi cholesterol-4-^{14}C						
		1 hr	4 hr	24 hr	3–4 days	5–7 days	11–13 days	17–19 days
G	Maternal	36*	29	1026	925			
	(Fetal)	(161,905)‡			(47,740)			
H	Maternal	41	98	382	1761			
	(Fetal)	(87,674)‡			(52,645)			
J	Maternal	40	35	303	686	598		
	(Fetal)	(697,515)‡				(17,542)		
K	Maternal	48	223	1859		1621	958	572
	(Fetal)	(24,373)‡						(12,689)
Mean ratio maternal to fetal serum SA					0.026	0.034		0.045

* The net counts of samples with SA 50 or below are the same as the control nonradioactive samples.
‡ The initial fetal samples were taken 5–10 min after fetal injection of the isotope, in order to verify the presence of cholesterol-4-^{14}C in the fetal circulation.

estimations of maternal-fetal transfer. According to the concept of the isotopic steady state, the ratio of cholesterol specific activities in maternal and fetal sera provides an index of the proportion of fetal cholesterol derived from maternal sources as cholesterol (4), provided the cholesterol flux is largely in the direction of mother to fetus. If all of the fetal cholesterol were transferred from the maternal blood the ratio should be unity. If fetal synthesis of cholesterol were also occurring there would be dilution of the labeled cholesterol from the mother reaching the fetus with a corresponding decrease in the ratio. In our five steady-state experiments, the ratio of fetal to maternal serum cholesterol SA averaged 42.6%. This was the proportion of fetal serum cholesterol originating by transfer from maternal blood. The remainder represents the minimal value resulting from fetal synthesis *de novo*. Approximately 40% of the cholesterol in fetal liver and heart was of maternal origin, while the amount derived from the mother was somewhat lower in the fetal lung, kidney, and gastrointestinal tract. The very low SA ratio of the fetal brain indicates that virtually all of the brain cholesterol was synthesized within that organ; brain cholesterol did not exchange appreciably with the fetal blood cholesterol. Clearly the blood-brain barrier is operative in the fetus with respect to cholesterol equilibration. As is indicated by these data, the monkey fetus can probably be compartmentalized in terms of at least three cholesterol pools—A, B, and C—as was demonstrated by Wilson in the adult primate (13).

When compared with similar studies in other animals,

TABLE VI

Concentration and Radioactivity of Maternal Serum Cholesterol during Pregnancy and Puerperium under Steady-State Conditions

Monkey No.	Serum cholesterol		SA serum cholesterol	
	Pregnancy*	Puerperium‡	Pregnancy*	Puerperium‡
	mg/100 ml		*dpm/mg*	
I	81±18.4	179±18.4	15,774±559	16,112±1,368
II	63±6.1	129±18.4	15,890±683	16,309±747
III	92±5.3	157±22.5	20,800±334	20,720±1,012
IV	93±7.4	176±14.6	10,934±1,074	10,120±570
V	90±8.9	189±14.6	16,174±803	15,464±755
Mean	84	166	15,914	15,745

* Mean±SD of weekly determinations throughout the last 4–6 wk antepartum.
‡ Mean±SD of weekly determinations throughout the first 6 wk post partum.

our finding that some 40% of the serum cholesterol in the term rhesus fetus is transferred from the mother suggests that considerable species variation exists. Previously reported values for the proportion of cholesterol in the term fetus derived from maternal sources as cholesterol include 15–20% in the rat (3) and 22% in the guinea pig (4). Thus, it appears that the monkey fetus derives a greater proportion of its cholesterol from maternal-fetal transfer than does the rodent. In view of the marked similarity between human and macaque in feto-placental physiology (14), it seems reasonable to postulate that a similar situation may occur in humans.

The hypothesis that amniotic fluid is derived from a secretion emanating from the placenta or umbilical cord is supported by the similarities of the amniotic fluid cholesterol SA to that of fetal serum. Clearly amniotic fluid could not have been derived from other fetal tissues having lower cholesterol SA.

The striking fall in maternal serum cholesterol during pregnancy in the rhesus monkey and its prompt rise to prepregnant levels within a week of parturition have been previously described (15, 16) and were confirmed in the present study. Similar though less marked effects have been reported in the baboon (17). The explanation of this change in serum cholesterol in subhuman primate pregnancy is unclear. In humans, the opposite situation occurs; the serum cholesterol increases during pregnancy and decreases post partum (18). The monkeys consumed a relatively low cholesterol diet (10 mg/day) suggesting that a difference in rate of intestinal absorption from dietary sources is unlikely. However, there could be differences in the absorption of endogenous cholesterol. Three other possible explanations remain. First of all, a continuous net transfer of cholesterol from mother to fetus during pregnancy could account for part of the change after pregnancy when this maternal loss would no longer be occurring. Secondly, cholesterol is used as a precursor in steroidogenesis by the feto-placental complex (19); after delivery hormone production falls and this extensive use of cholesterol no longer occurs. A third explanation probably lies in the dilution of maternal cholesterol pools by increased tissue mass and extracellar spaces. An increase in blood volume would account for part of this but other factors, such as the placenta, the conceptus, and the pregnant uterus, must be involved as well. All of these growing organs have a need for structural cholesterol which comes in part from maternal plasma. In the puerperium these needs no longer exist. The myometrium undergoes remarkable regression, resulting in a marked reduction in its total cholesterol content. This excess cholesterol thus may enter the body's labile pools.

Unexpected was the finding that the serum cholesterol SA did not change when the serum cholesterol doubled during the puerperium. Ordinarily, with a virtually cholesterol-free diet and a presumably constant intake of isotopic cholesterol, a doubling of the serum cholesterol level, if from nonlabeled sources, would have reduced the serum cholesterol SA by 50%. Since no reduction in SA occurred, the increased number of labeled cholesterol molecules (their plasma number must have doubled also) must have been derived from previously labeled tissue cholesterol such as the gravid uterus and the mammary glands which would be undergoing profound regression in the puerperium. A contraction of the blood volume after delivery also occurs. This process could also supply labeled cholesterol of a high SA.

TABLE VII

Cholesterol Concentration and Radioactivity of Breast Milk under Steady-State Conditions

Monkey No.	Cholesterol	SA	SA of milk as per cent of maternal serum SA
	mg/100 ml	*dpm/mg cholesterol*	
I	40	12,750	76.8
II	19	10,750	64.5
III	51	15,510	73.3
IV	41	7,429	65.5
V	21	9,630	58.7
Mean	35		67.8

The cholesterol concentration in breast milk obtained by expression on the 2nd day after delivery varied from 19 to 51 mg/100 ml, values which are similar to reported levels in human milk. Comparison of cholesterol specific activities of milk and serum indicated that approximately two-thirds of milk cholesterol resulted from transfer from maternal blood, with the remainder presumably originating from mammary gland synthesis (2, 20). Comparable reported values for the proportion of milk cholesterol derived from maternal blood include 80 and 83% in the rat (2, 21) and 28–45% in the guinea pig (22).

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