Placental transfer of cholesterol-4-^{14}C into rabbit and guinea pig fetus

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ABSTRACT A tracer dose of cholesterol-4-^{14}C was given daily in the diet of six pregnant guinea pigs to establish an isotopic steady state. At the time of parturition, maternal and fetal blood and fetal tissues were collected and analyzed for cholesterol content and cholesterol specific activity. A comparison of these specific activities in neonatal and maternal serum indicated that about 22% of the fetal serum cholesterol was transferred from maternal blood. In the newborn, tissues generally had the same cholesterol specific activity as serum. Brain tissue was an exception in having a specific activity only 8.4% of that of serum. Dietary cholesterol did not increase serum cholesterol levels in the newborn but did increase the percentage of fetal cholesterol derived from the maternal circulation. The rapid transfer of cholesterol-4-^{14}C across the placenta was indicated by the appearance of this isotope in the newborn 2 days after its administration to pregnant rabbits. A considerable amount of the cholesterol content of newborn guinea pigs and rabbits originated from the maternal blood.

KEY WORDS cholesterol-4-^{14}C · metabolism · stability · dietary cholesterol · isotopic steady state · pregnancy · fetal cholesterol · origin · synthesis · newborn · rabbit · guinea pig

THE POSSIBLE EXCHANGE of lipids from the maternal to fetal circulation has intrigued investigators for many years. In 1935, Boyd and Wilson deduced that free cholesterol passed from the placenta to the human fetus after they had found a higher cholesterol value in umbilical vein blood than in umbilical artery blood (1). Later Goldwater and Stetten found that deuterium-labeled cholesterol passed from maternal to fetal blood in rats (2). In 1950, however, Popják and Beeckmans concluded that there was no perceptible transfer of cholesterol from the mother to the fetus and that fetal cholesterol resulted from synthesis within the body of the fetus (3). These conclusions were based upon analysis of fetal and maternal tissues after the administration of deuterium oxide and ^{14}C-labeled acetate to pregnant rabbits. In 1965, Connor, Osborne, and Marion found that the laying hen transferred cholesterol-4-^{14}C from the blood across the ovarian membranes into developing egg yolks (4). Recently Chevallier produced an isotopic steady state in pregnant rats by feeding cholesterol-4-^{14}C and noted that 60–70% of the cholesterol in the 12 day old fetus is of maternal origin (5). This value decreased to 15–20% by the time of parturition.

Because of these apparently divergent results, we made further studies to determine whether placental transfer of cholesterol into the fetus did occur in other species, and, if transfer could be demonstrated, to examine the incorporation of cholesterol into various fetal tissues. Two types of studies were performed. Cholesterol-4-^{14}C was given in a single dose by gavage to pregnant rabbits. In another experiment, we fed a colony of guinea pigs cholesterol-4-^{14}C in the chow to produce an isotopic steady state before and during pregnancy. The effect of dietary cholesterol upon cholesterol concentration in maternal blood and in the blood and tissues of the newborn was also examined.

METHODS AND MATERIALS

Single dose study

14–50 μc of cholesterol-4-^{14}C (New England Nuclear Corp, Boston, Mass.) in 0.5 ml of ethanol was given by gavage in a single dose to four pregnant rabbits at different times before parturition. They were fed standard laboratory rabbit chow, which is cholesterol-free (Purina

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Rabbit Chow, Ralston Purina Co., St. Louis, Mo.). Venous blood was collected at intervals during pregnancy and after parturition. The newborn rabbits were anesthetized with ether and blood was drawn by cardiac puncture immediately after birth. They were then killed with ether and the various organs were collected for analysis. The cholesterol content of the serum was determined by the Abell, Levy, Brodie, and Kendall method (6).

For the determination of radioactivity, serum was saponified with alcoholic KOH. The nonsaponifiable residue was extracted with hexane, dried, and dissolved in 10 ml of scintillation mixture (4 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene in 1 liter of toluene). These samples were counted in a Packard Tri-Carb liquid scintillation spectrometer with an efficiency of 58%. The results were expressed as specific activity in cpm/mg of cholesterol. The tissues of different organs were dried under vacuum at 100°C, ground to a powder, and extracted with chloroform–methanol (7). The cholesterol and cholesterol-4-^{14}C contents of the tissue extracts were then determined by the methods already described.

Steady State Study

Adult female guinea pigs (weight 500–700 g) were raised in the same cage with an adult male guinea pig which served as a boar. Radioactive cholesterol diets were prepared by mixing guinea pig chow (Yoder Company, Kalona, Iowa) with an ether solution of cholesterol-4-^{14}C and peanut oil. In some diets, nonradioactive cholesterol was added (Table 1). The ether solvent was evaporated subsequently. This special diet was then fed ad libitum to all guinea pigs. The period of cholesterol-4-^{14}C feeding ranged from 2 to 5.5 months. In order to observe the establishment and maintenance of the radioactive steady state in these female guinea pigs, we collected blood samples periodically and determined the cholesterol specific activities.

When a female guinea pig developed the signs of pregnancy, it was placed in an individual cage and fed the same radiolabeled diet. Within 12 hr after parturition, the blood of both dam and piglets was collected. Blood from the newborn was obtained by cardiac puncture. Maternal blood was drawn from a paw vein. Specimens of different organs of the newborn piglets were analyzed by the same methods described for rabbits. After parturition, the maternal guinea pig was returned to the original cage with the boar for further mating. Guinea pigs I and II became pregnant twice.

In order to demonstrate that the radioactivity in the tissue extract was confined to its cholesterol content, we subjected the lipids in the tissue extract to thin-layer chromatography. The lipids were chromatographed on a glass plate (20×20 cm) coated with Silica Gel G 0.5 mm thick. The solvent system was hexane–chloroform–ethyl ether–acetic acid 80:10:10:1. The band of each individual lipid was scraped off the plate and the absorbed lipid was eluted with chloroform into counting vials. The radioactivity of the various lipid bands was then determined.

TABLE 1 Composition of Special Diets

Constituents	Diet A	Diet B
Guinea pig lab chow (*g*)	100	100
Peanut oil (*g*)	0.5	0.5
Cholesterol-4-^{14}C (μc)	0.67	0.67
Nonradioactive cholesterol (% of diet)	0	0.10

That the sterols in the cholesterol and cholesterol ester bands of maternal and fetal serum and liver were actually cholesterol (5-cholesten-3β-ol) was established as follows. The eluents of the thin-layer chromatography plates were hydrolyzed to the free form by saponification, converted to the trimethylsilyl ether derivatives of the sterol, and subjected to gas–liquid chromatography. The analyses were performed on an instrument equipped with a hydrogen flame ionization detector (F & M biochemical gas chromatograph, model 400, Avondale, Pa.). The column was a 120 cm glass U-tube, 4 mm I.D., packed with Diatoport S (80–100 mesh) coated with a 3.8% film of SE-30 (methylsiloxane polymer). Temperatures of column, detector, and flash heater were 230, 250, and 300°C respectively. Helium was used as carrier gas at a flow rate of 100 ml/min; the inlet pressure was 40 psi.

The purity of the administered cholesterol-4-^{14}C was verified by thin-layer chromatography as described by Mangold (8). The radioactivity was present in the cholesterol band only.

RESULTS

Single Dose Study

In the pregnant rabbits given cholesterol-4-^{14}C in a single dose 8–18 days before parturition, there was evidence that a considerable amount of this isotope was transferred into the fetus (Table 2). In rabbit I, for example, the serum cholesterol specific activities of the five newborn rabbits ranged from 940 to 1420 cpm/mg compared with a maternal serum specific activity of 4040. Tissue cholesterol specific activities were generally similar to those of sera in the newborn. These included liver, heart, lung, kidney, and skeletal muscle. Brain, on the other hand, had specific activities of 297–382 in different newborn rabbits, or about 30% of the newborn serum values. The data from rabbit II, given the same dose of cholesterol-4-^{14}C at the same time (18 days) before parturition, led to the same conclusions. In rabbit III, given a dose of isotope 8 days before parturition, significant transfer of cholesterol-4-^{14}C occurred. Brain specific activity was

TABLE 2 COMPARISON OF RADIOACTIVITY AND CHOLESTEROL CONTENT OF MATERNAL SERUM AND NEONATAL SERUM AND TISSUES IN THE RABBIT (SINGLE DOSE STUDY)

Rabbit No.*		Serum		Liver		Heart		Lung		Kidney		Muscle		Brain	
Mother	Newborn	SA†	Cholesterol	SA	Chol.‡	SA	Chol.	SA	Chol.	SA	Chol.	SA	Chol.	SA	Chol.
			mg/100 ml												
I		4040	79												
	I-1	1080	180	1013	9.8	1155	10.2	1211	21.0	1540	13.4	1569	9.5	316	41.6
	I-2	940	93			1123	10.2	1184	20.6	1734	15.4	1641	8.8	297	41.8
	I-3	1260	158	1108	12.3	1192	14.1	1438	18.6	1060	19.9	968	10.3	323	48.1
	I-4	1420	140	1210	12.7	1397	10.6	1484	19.9	1225	17.3	1233	6.8	382	41.8
	I-5	1150	114	1337	11.2	1109	10.7	1471	20.7	1387	15.1	1128	10.3	314	41.9
II		4280	36												
	II-1			1294	8.9			1549	18.9	1298	12.4	1313	6.8	211	40.3
	II-2	1140	87	949	12.0	1356	11.2	1284	18.8	1023	13.2	965	11.2		
	II-3	1170	118	1159	9.7			1308	10.9	1006	15.6	1241	11.5	194	39.8
	II-4			996	14.9	1343	10.5	1437	11.2	844	13.2	1024	19.7	148	34.1
	II-5	1360	130	1350	11.7	1600	10.4	1818	18.3	1310	13.5	1440	8.7	240	38.8
	II-6	1310	130	1729	10.6			1605	18.5	1362	9.0	1222	9.7		
	II-7	1210	126	1160	6.8	1642	9.2	1429	26.6	1141	16.1	633	7.8	146	41.9
III		550	33												
	III-1	274	91	320	10.4	364	11.7	386	22.1	485	15.4	333	7.8	37	38.2
	III-2			320	7.1	375	12.2	356	16.6	333	18.4	267	9.4	44	26.1
	III-3			276	6.3	308	8.4	273	14.7	267	15.7	202	10.0	35	29.0

* Rabbits I and II were given 50 μc of cholesterol-4-^{14}C 18 days before parturition. Rabbit III was given 14 μc of cholesterol-4-^{14}C 8 days before parturition.
† Specific activity (SA) is expressed as cpm/mg of cholesterol.
‡ Tissue cholesterol (Chol.) expressed as mg/g of dried tissue.

even relatively lower—14% of the serum specific activity for newborn no. III-1. Even in a rabbit injected 2 days before parturition, cholesterol-4-^{14}C was found in blood and tissues of the newborn, which indicates rapid transfer across the placenta and incorporation into the fetus. The specific activity of neonatal serum cholesterol was 225 cpm/mg versus a maternal value of 21,800. Tissue cholesterol specific activities ranged from 90 to 180.

Steady State Study

Consecutive serum specific activities in the guinea pigs fed a constant amount of cholesterol-4-^{14}C in the diet indicated that the isotopic steady state was reached for all animals 5–6 wk after the beginning of cholesterol-4-^{14}C feeding (Fig. 1). The serum cholesterol specific activity was thus constant throughout most or all of pregnancy.

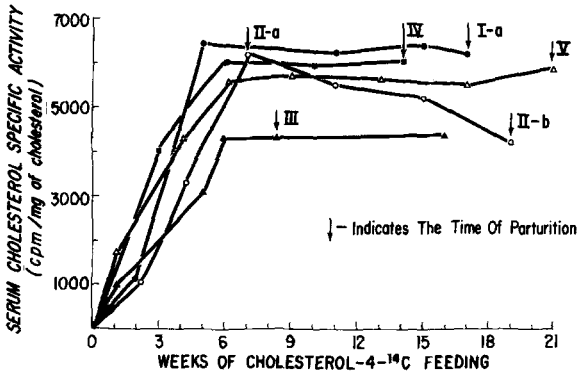

FIG. 1. Establishment of an isotopic steady state in five pregnant guinea pigs. The curves of serum cholesterol specific radioactivity generally reached a plateau after 5–6 wk of feeding cholesterol-4-^{14}C.

An attempt was made to study the effect of dietary cholesterol on maternal and fetal serum cholesterol levels and on the degree of cholesterol transfer from mother to fetus. Some of the guinea pigs were later fed the same cholesterol-4-^{14}C diet with 0.1% nonradioactive cholesterol added. Guinea pigs I and II became pregnant twice. Guinea pig I had one pregnancy during the cholesterol-free diet period and another pregnancy during the 0.1% cholesterol diet period. Because of the dilution of exogenous cholesterol, the serum cholesterol specific activity of this guinea pig decreased from 6220 to 2130 between the two parturitions. Guinea pig II was pregnant twice in the same cholesterol-free diet period. For uncertain reasons, its serum specific activity dropped from 6220 to 4160 between the two parturitions, a 12-wk interval, while its serum cholesterol remained relatively constant.

The cholesterol concentrations and specific activities of serum and various organs of the isotopic steady state guinea pigs and their offspring are listed in Tables 3 and 4. The cholesterol-4-^{14}C fed to the mother was, clearly, transferred into the blood and tissues of the newborn. In general, for the guinea pigs given only the tracer dose of cholesterol-4-^{14}C, the neonatal sera had cholesterol specific activities ranging from 13 to 30% of the specific activity of maternal serum (Table 3). For example, Ia had a serum cholesterol specific activity of 6200; its two offspring, 1660 and 1410 respectively. The mean percentage of neonatal to maternal serum specific activity for all guinea pigs was 21.5% (Table 5). Liver, heart, and lung of the newborn guinea pigs had cholesterol specific activities very similar to that in the corresponding serum (Tables 3 and 6), kidney and muscle gave about 75% of

TABLE 3 COMPARISON OF THE RADIOACTIVITY AND CHOLESTEROL CONTENT OF MATERNAL SERUM AND NEONATAL SERUM AND TISSUES IN THE GUINEA PIG (STEADY STATE ANIMALS)

Guinea Pig No.		Serum		Liver		Heart		Lung		Kidney		Muscle		Brain	
Mother*	Newborn	SA†	Cholesterol	SA	Chol.‡	SA	Chol.	SA	Chol.	SA	Chol.	SA	Chol.	SA	Chol.
			mg/100 ml												
Ia		6200	55												
	Ia-1	1660	86	1578	5.9	1357	8.6	1296	20.8	1080	6.6	912	4.3	122	67.2
	Ia-2	1410	87	1504	5.6	1057	8.7	1373	16.4	1086	5.9	939	3.9	112	65.1
IIa§		6220	54												
	IIa-1	800	230	926	15.9	921	10.5	826	24.3	737	12.2	658	5.0	67	66.7
	IIa-2	800	196	966	14.7	940	9.4	838	22.5	—	—	675	5.4	77	67.8
	IIa-3	1010	221	1072	19.1	880	11.1	895	18.2	755	21.0	1145	5.0	78	69.0
IIb		4160	38												
	IIb-1	985	110	1230	8.4	1070	9.0	1120	20.7	762	20.4	770	3.5	97	67.5
	IIb-2	985	120	1040	9.0	630	8.9	960	20.4	776	17.7	625	4.4	97	66.0
III		4260	72												
	III-1	990	60	1348	6.3	1200	9.1	1242	20.4	816	9.6	789	4.4	104	66.1
	III-2	1279	60	1304	6.3	1200	9.6	1208	21.6	961	7.5	681	4.8	88	70.3
	III-3	1090	69	1481	6.2	1263	9.4	1369	21.6	994	15.8	889	4.9	91	75.6
IV		6100	60												
	IV-1	990	92	1260	6.3	1115	8.2	1169	14.9	897	9.4	751	4.7	79	63.2
	IV-2	1160	115	1127	7.2	1005	8.2	1048	18.3	784	6.7	616	5.2	89	64.4
V		5980	62												
	V-1	1640	75	1348	9.0	1388	8.4	1150	20.1	1112	8.5	900	4.6	118	67.1
	V-2	1290	82	1343	9.4	1135	8.9	1170	20.1	1061	6.4	969	4.8	115	70.2

* The maternal diet was cholesterol free, with a constant intake of cholesterol-4-^{14}C (Table 1).
† Specific activity (SA) is cpm/mg of cholesterol.
‡ Tissue cholesterol (chol.) expressed as mg/g of dried tissue.
§ Guinea pig II became pregnant and delivered piglets on two occasions, noted as a and b.

TABLE 4 EFFECT OF DIETARY CHOLESTEROL UPON THE SERUM AND TISSUE CHOLESTEROL CONTENT AND SPECIFIC RADIOACTIVITY IN THE NEWBORN GUINEA PIG

Guinea Pig No.		Serum		Liver		Heart		Lung		Kidney		Muscle		Brain	
Mother	Newborn	SA*	Cholesterol	SA	Chol.†	SA	Chol.	SA	Chol.	SA	Chol.	SA	Chol.	SA	Chol.
			mg/100 ml												
Ib		2130	285												
	Ib-1	1210	85	1471	8.6	1330	7.5	1387	16.4	1203	17.3	1167	5.6	190	53.5
	Ib-2‡	—	—	1764	14.2	1762	13.5	1526	25.9	1431	24.1	1276	7.4	—	—
VI		3520	237												
	VI-1	1680	172	2065	7.5	2378	8.3	2285	19.9	1110	22.7	1600	4.7	215	55.3
	VI-2	1840	142	2034	8.6	2245	7.2	2309	14.2	1405	24.7	1625	6.1	254	48.1

The maternal diet contained 0.1% nonradioactive cholesterol in additions to the tracer dose of cholesterol-4-^{14}C.
* Specific activity is expressed as cpm/mg of cholesterol.
† Tissue cholesterol (chol.) is expressed as mg/g of dried tissue.
‡ Born dead; no serum could be obtained.

TABLE 5 EVIDENCE FOR MATERNAL ORIGIN OF CHOLESTEROL IN TISSUES OF THE NEONATAL GUINEA PIG

	Maternal Diet	
	Cholesterol-Free	0.1% Cholesterol
Number of newborn guinea pigs	14	3
Serum	21.5 ± 2.3%	52.3 ± 4.5%
Liver	24.0 ± 6.3	62.0 ± 6.1
Heart	20.0 ± 5.7	65.0 ± 3.1
Lung	21.0 ± 6.1	65.0 ± 0.7
Kidney	17.0 ± 3.6	43.0 ± 12.2
Muscle	15.0 ± 3.3	49.0 ± 5.5
Brain	1.8 ± 0.5	7.4 ± 1.4

The specific activities of tissues of the newborn are expressed as percentages of the specific activity of maternal serum. P was <0.01 for every difference between the two diets measured.

this value, and brain only 8%. For example, brain specific activity was only 122 and 112 for the offspring of guinea pig Ia, whose serum values were 1660 and 1410.

In the guinea pigs fed 0.1% cholesterol, the serum specific activity of the newborn was considerably higher relative to the maternal serum specific activity than for guinea pigs born from mothers consuming a cholesterol-free diet (Table 4). Thus the apparent ratio of newborn to maternal specific activities was increased. For guinea pig Ib, this was 1210:2130, or 57%. The tissue cholesterol specific activities also tended to be somewhat higher relative to serum in the offspring of cholesterol-fed versus noncholesterol-fed guinea pigs (Table 6).

Because some guinea pigs gave birth during the night, and the piglets remained with the mother for several

TABLE 6 SPECIFIC ACTIVITIES OF NEONATAL TISSUE COMPARED TO THAT OF NEONATAL SERUM

	Maternal Diet		
	Cholesterol-free	0.1% Cholesterol	*P* for Difference
Number of newborn guinea pigs	14	3	
	% of neonatal serum SA		
Liver	111.0 ± 16.0	118.0 ± 6.4	<0.3
Heart	97.0 ± 18.0	124.0 ± 14.0	<0.02
Lung	100.0 ± 16.0	125.0 ± 10.0	<0.01
Kidney	79.0 ± 10.0	80.0 ± 17.0	<0.9
Muscle	73.0 ± 16.0	93.0 ± 4.0	<0.01
Brain	8.4 ± 1.2	14.1 ± 2.1	<0.01

hours, there was a possibility that the newborn piglets might obtain radioactive cholesterol from the ingestion of milk from the lactating dam since cholesterol-4-^{14}C is transferred into the milk from the maternal blood (W.E. Connor and D. S. Lin, 8*a*). Therefore, the stomachs of six piglets (one from each of six pregnancies) were examined. Two of them were found empty and four contained a white curd-like material which was analyzed for cholesterol content and radioactivity. The radioactivity of this material was much lower than that of newborn serum (on the average, the specific activities were 729 vs. 1860).

Tissue and Serum Cholesterol Content

For both guinea pigs and rabbits, the serum and tissue cholesterol content varied widely. On the cholesterol-free diet, newborn guinea pigs had higher serum cholesterol levels than the mothers (Table 3). This situation was reversed by the 0.1% cholesterol diet (Table 4). The average serum cholesterol concentration of maternal guinea pigs fed the cholesterol-free diet was 57 mg/100 ml and that of the newborn guinea pigs was 115 mg/100 ml (Table 7). In contrast, for the maternal guinea pigs fed 0.1% cholesterol diet, the serum cholesterol level was 261 mg/100 ml; the newborn level was 133 mg/100 ml. The cholesterol content of some newborn tissues (liver, heart, lung, and muscle) did not change with the addition of cholesterol to the maternal diet. Others had a statistically significant change in cholesterol content. Kidney cholesterol increased from 11 to 23 mg/g of dried weight.

The mean serum cholesterol content of 3 female adult rabbits fed cholesterol-free diets was 49 mg/100 ml and that of 15 newborn rabbits was 124 mg/100 ml (Table 7). The mean cholesterol content per g of tissue in the newborn was 10 mg for liver, heart, and muscle; 15 mg for kidney; 19 mg for lung; and 39 mg for brain (Table 7). In both newborn guinea pigs and rabbits, the brain had the highest cholesterol content of any tissue (40–70 mg/g versus 5–20 mg/g for other tissues).

Localization of Radioactivity in Lipids of Various Organs

The lipid extracts of neonatal and maternal serum and liver were fractionated into phospholipids, cholesterol, triglycerides, and cholesteryl esters by thin-layer chromatography. The radioactivity of each band was determined by the method described earlier. The analysis of two maternal sera and livers, two fetal sera, and six fetal livers demonstrated that over 95% of the radioactivity was contained in the cholesterol and cholesteryl ester bands. This result suggested that the radioactivity was confined only to the sterol nucleus, which was transferred intact from the diet, through the maternal blood and tissues, across the placenta into the fetal blood and tissues.

The lipids in the eluates of the cholesterol bands from thin-layer plates were further identified as only cholesterol by gas–liquid chromatography. Single peaks with the same retention time as that of a pure cholesterol standard were found.

TABLE 7 MEAN SERUM AND TISSUE CHOLESTEROL CONCENTRATION OF MATERNAL AND NEWBORN GUINEA PIGS AND RABBITS

	Guinea Pigs	Guinea Pigs	Rabbits
Nonradioactive cholesterol in diet (%)	0	0.1	0
Number of mothers	6	2	3
Number of newborn	14	3	15
		mg/100 ml	
Maternal serum	57.0 ± 11.3	261.0 ± 34.0	49.0 ± 25.7
Newborn serum	115.0 ± 59.6	133.0 ± 44.0	124.0 ± 28.6
		mg/g of dried tissue	
Liver	9.2 ± 5.3	9.7 ± 3.0	10.3 ± 2.4
Heart	9.2 ± 0.8	9.1 ± 2.9	10.8 ± 1.5
Lung	20.0 ± 2.4	19.1 ± 4.8	18.5 ± 4.0
Kidney	11.4 ± 7.0	22.9 ± 6.0	14.8 ± 2.6
Muscle	4.6 ± 0.5	5.9 ± 1.1	9.9 ± 3.1
Brain	67.6 ± 4.1	52.2 ± 3.7	38.7 ± 5.9

DISCUSSION

In both the single dose and steady state studies, cholesterol was transferred from maternal circulation into the fetal blood, as evidenced by the high level of cholesterol-4-^{14}C in the blood of the newborn. The appearance of cholesterol-4-^{14}C in newborn tissues two days after its administration suggested a rapid transfer of cholesterol from maternal blood to fetal blood and into the various fetal organs. The newborn tissues, except for brain, were similar in specific activities to the newborn serum.

In the isotopic steady state study it was assumed that cholesterol-4-^{14}C was constantly moving from the maternal blood into the fetal blood and thence into the developing tissues of the fetus. If fetal cholesterol were completely derived from the maternal blood, then the ratio of newborn and maternal specific activities should be unity. If fetal cholesterol were partly derived from synthesis, the labeled cholesterol of maternal origin would be correspondingly diluted: the ratios of neonatal and maternal specific activities provide an index of the amount of fetal cholesterol that was derived from maternal blood. In the guinea pigs receiving a cholesterol-free diet, 20–24% of the cholesterol in the serum, liver, heart, and lung of the newborn guinea pig was of maternal origin; in the kidney and skeletal muscle, 15–17%; and in the brain, only 1.8%.

With 0.1% cholesterol in the maternal diet and with consequently higher maternal serum cholesterol concentrations, neonatal cholesterol seemed by this criterion to be derived to a greater extent from the maternal blood. Thus, 52–65% of neonatal serum, liver, heart, and lung cholesterol appeared to be derived from maternal serum; 43 and 49% of kidney and muscle cholesterol; and 7.4% of brain cholesterol. The neonatal serum cholesterol concentration remained unchanged while that of maternal blood cholesterol increased greatly after the addition of dietary cholesterol (Table 7). It therefore seemed likely that cholesterol in the maternal diet increased the amount of cholesterol transferred from maternal blood to fetal blood, perhaps through a higher rate of transfer. The increased transfer may have suppressed cholesterol synthesis in the fetus, possibly through feedback inhibition, so that more of the fetal cholesterol was derived from maternal sources.

In the newborn guinea pig, the ratio of tissue cholesterol specific activity to that of serum indicated the rate and degree of exchange between tissue and serum. In some organs such as liver, heart, and lung, cholesterol appeared to be in equilibrium with the blood as indicated by specific activity ratios approaching unity. The specific activity ratio suggested that very little brain cholesterol was derived from or exchanged with fetal blood cholesterol (8.4% only). Waelsch, Sperry, and Stoyanoff (9) and LeRoy (10) reported that adult brain does not synthesize cholesterol, which in the nervous tissue is a structural element (11). Our results suggested that most, but not all, of the brain cholesterol was synthesized in the brain and did not exchange appreciably with the cholesterol in blood.

When the maternal rabbits and guinea pigs were fed the cholesterol-free diet, their serum cholesterol concentrations were lower than those of the newborn. These results agree with the report of Popják that the lipids in fetal plasma are usually at a lower level than in maternal blood except for rabbits and guinea pigs (12). Dietary cholesterol increased the maternal serum cholesterol of guinea pigs significantly but not that of neonatal blood and tissues; Friedman also reported that the serum cholesterol of the rabbit fetus was not affected by an increased serum cholesterol level in the mother rabbit (13).

In spite of different conclusions of various authors about placental cholesterol transfer, we believe that no basic conflict exists. Popják and Beeckmans administered deuterium oxide and acetate-^{14}C to pregnant rabbits and compared deuterium content in maternal plasma or liver and placenta with that in fetal liver and carcass lipids (3). Since the ^{2}H content of cholesterol in placenta was less than that in either the fetal liver or fetal carcass, they concluded that the cholesterol of the fetus could not have been derived via placenta from maternal plasma, but must have been synthesized within the fetus. Since the fetal serum cholesterol level is higher than that of the mother, an alternative interpretation might have been that the fetal cholesterol could represent both the cholesterol synthesized by fetus and cholesterol synthesized by the mother and then transferred to fetus via the placenta.

Chevallier (5) reported that, after an isotopic steady state had been produced in female rats by the feeding of cholesterol-4-^{14}C, 15–20% of the cholesterol in the fetus was transferred from maternal blood. This result is similar to what we obtained in two other species. He also reported that, in 12–13-day old fetuses, 60–70% of cholesterol was of maternal origin. Roux (14) found the incorporation of acetate-1-^{14}C into hepatic cholesterol of the 25 and 30 day old rabbit fetus was $^{1}/_{2}$ to $^{1}/_{3}$ the incorporation into the 20 day old fetus. Since the fetus is growing more rapidly during the later part of gestation, it is conceivable that both increased transfer of cholesterol from the mother and rapid fetal synthesis are needed to meet sterol requirements.

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References

1. Boyd, E. M., and K. M. Wilson. 1935. *J. Clin. Invest.* **14:** 7.
2. Goldwater, W. H., and D. Stetten, Jr. 1947. *J. Biol. Chem.* **169:** 723.
3. Popják, G., and M. Beeckmans. 1950. *Biochem. J.* **46:** 547.
4. Connor, W. E., J. W. Osborne, and W. L. Marion. 1965. *Proc. Soc. Exptl. Biol. Med.* **118:** 710.
5. Chevallier, F. 1964. *Biochim. Biophys. Acta.* **84:** 316.
6. Abell, L. L., B. B. Levy, B. B. Brodie, and F. F. Kendall. 1952. *J. Biol. Chem.* **195:** 357.
7. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. *J. Biol. Chem.* **226:** 497.
8. Mangold, H. K. 1961. *J. Am. Oil Chemists' Soc.* **38:** 708.

8*a*. Conner, W. E., and D. S. Lin. *Am. J. Physiol.* In press.

9. Waelsch, H., W. M. Sperry, and V. A. Stoyanoff. 1940. *J. Biol. Chem.* **135:** 297.
10. LeRoy, G. V. 1956. *Ann. Int. Med.* **44:** 524.
11. Finean, J. B. 1956. *In* Biochemical Problems of Lipids. G. Popják and E. le Breton, editors. Butterworths Scientific Publications, London. 127.
12. Popják, G. 1954. *Cold Spring Harbor Symp. Quant. Biol.* **19:** 200.
13. Friedman, M., and O. B. Sanford. 1961. *Am. J. Physiol.* **201:** 611.
14. Roux, J. F. 1966. *Metabolism.* **15:** 856.