Placental transport of free palmitic and linoleic acids in the guinea pig

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ABSTRACT Radioisotopic tracers were used to measure the unidirectional transfer rates of free fatty acids across the placenta of fed and fasted pregnant guinea pigs. Free ^{14}C-labeled palmitic and linoleic acids (in serum) were injected simultaneously into a jugular vein of an anesthetized pregnant guinea pig. Serial samples of maternal blood were collected from a carotid artery; fetal blood was collected from the umbilical vein of an exposed fetus. Analysis of maternal and fetal plasma revealed that: (*a*) the half-lives of free palmitic and linoleic acid in maternal plasma are approximately 1.3 min and 0.7 min, both in fed animals with low plasma concentrations of these acids and in fasted animals with high concentrations; (*b*) free linoleic and palmitic acids cross the placenta from maternal to fetal plasma in a ratio of approximately 2.0, a value which appears not to change as the transfer rates of these acids from maternal to fetal plasma are increased by fasting the mother. It is suggested that the ratio in which free linoleic and palmitic acids cross the placenta from maternal to fetal plasma is determined by the ratio of the *unbound* free linoleic and palmitic acid concentrations in maternal plasma.

A comparison of several species indicates that a much greater proportion of fetal fatty acids comes from the mother in the guinea pig and rabbit than in the rat, the sheep, or man.

KEY WORDS placental transport · palmitic acid · linoleic acid · guinea pig · species differences · plasma FFA · half-life · turnover rate · blood sampling · exposed fetus

LARGE AMOUNTS OF FATTY ACIDS are required for the growth and development of the mammalian fetus. The nonessential fatty acids such as myristic, palmitic, stearic, palmitoleic, and oleic can be synthesized in fetal tissues (1); the essential polyunsaturated fatty acids such as linoleic and linolenic must come from the mother, being derived solely from the maternal diet. Evidence in a number of species (2–4) suggests that the esterified fatty acids of maternal plasma do not normally cross the placental barrier in anything more than trace amounts. However, the FFA of maternal plasma may be an important source of fetal fatty acids, at least in the guinea pig (3) and rabbit (5) where palmitic acid-^{14}C injected into the maternal circulation rapidly appears in the fetus in significant quantities.

In the present study we have measured, with radioisotopic tracers, the rates of transfer (equivalents/min) of free palmitic and linoleic acids across the guinea pig placenta from maternal to fetal plasma. The half-lives of these acids in maternal plasma, as well as their concentrations in both maternal and fetal plasma, were determined. The preparation devised for the study of placental transport in the guinea pig should be of general use; it is probably as close to a normally functioning system as can be conveniently achieved in a small laboratory animal.

METHODS

Animals and Diet

Pregnant and nonpregnant albino guinea pigs of the Hartley strain were purchased from the Camm Research Institute, Wayne, N.J. Fetal age was estimated on the basis of weight and crown–rump length (6).

Guinea pigs at the Camm Research Institute are fed a diet of green vegetables and pellets made from 40% alfalfa leaf, 34% whole wheat, 15% soybean meal, 5% tomato pomace, 2% brewer's yeast, 1% corn oil, 1% NaCl, 1% $CaCO_3$, and 1% bone meal. The pellets contain 16% protein and 2.5% lipid. The composition of total fatty acid in the pellets was found to be 1% myristic, 23% palmitic, 1% palmitoleic, 3% stearic, 17% oleic, 40% linoleic, and 15% linolenic.

Abbreviations: FFA, free fatty acid(s); TLC, thin-layer chromatography; GLC, gas–liquid chromatography; dimethyl POPOP, 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene; PPO, 2,5-diphenyloxazole. Fatty acids designated by number of carbon atoms:number of double bonds.

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Plasma FFA Concentration

FFA was measured by titration (7) in plasma extracts prepared by the procedure of Dole and Meinertz (7) as modified by Trout, Estes, and Friedberg (8); duplicate measurements agreed within 5%. Titrated acid was shown to be FFA by TLC.

Plasma FFA Composition

FFA was isolated from plasma extracts (7) by TLC on Silica Gel G (Brinkmann Instruments Inc., Westbury, N.Y.) with hexane–diethyl ether–acetic acid 82:18:1. The silica gel was sprayed with 0.2% 2′,7′-dichlorofluorescein, after which the FFA zone was viewed under UV light, scraped onto glassine paper, and transferred to a Pasteur pipette plugged with glass wool. The fatty acids were then eluted with 6–8 ml of diethyl ether, methylated with freshly distilled diazomethane at 0°C (9), and separated by GLC in a Pye argon chromatograph (Pye Instruments, Cambridge, England) fitted with an ionization detector. The gas phase of argon was at a pressure of 8 psi; the stationary phase consisted of (by weight) 17.5% ethylene glycol succinate polyester on Gas-Chrom P (Applied Science Laboratories Inc., State College, Pa.) packed in a 4 ft long column. Analyses were carried out at 167°C. Standard mixtures of methyl esters of fatty acids (obtained from the Applied Science Laboratories, Inc.) were used to identify the peaks; the areas beneath the peaks were estimated by triangulation to determine the relative amounts of each acid. Composition has been expressed on a molar basis throughout (Tables 1, 5–7, below). Duplicate analyses consistently agreed within 1–2%. Quantitative results with fatty acid standards obtained from Applied Science Laboratories (Mixtures C and D) agreed with the stated composition data with a relative error of less than 5% for major components (more than 10% of total mixture) and less than 8% for minor components (less than 10% of total mixture).

Lipid Concentration and Total Fatty Acid Composition in Plasma, Adipose Tissue, and Liver

Lipid was extracted from a tissue with 19 volumes of $CHCl_3$–CH_3OH 2:1; the extract was filtered, washed with $^1/_5$ volume of water, and clarified by the addition of a small amount of methanol. The concentration of lipid in an aliquot of the extract was determined gravimetrically after evaporation of the solvent. The composition of total fatty acid was determined by GLC after saponification with 10% methanolic KOH and diazomethylation of the fatty acids. Early samples were analyzed on a 3% Apiezon L column at 193°C which did not separate linoleic and linolenic acids; for these analyses only the sum of these two acids is reported (Tables 5–7, below).

Radiochemical Purity of Fatty Acids

Palmitic acid-^{14}C (15 μc/μmole) and linoleic acid-^{14}C (15 μc/μmole) were purchased from Applied Science Laboratories. The radiochemical purity of these materials, examined by GLC and also by argentation TLC, was 98% and 93%, respectively.

Preparation of Fatty Acids for Intravenous Injection

Radioactive palmitic and linoleic acids were prepared for experimental use by suspending each acid in 1.0 ml of ethanol, 1.5 ml of 0.01 N NaOH, and a trace of Tween 20, then evaporating the ethanol at 40°C under N_2. The resulting clear stock mixtures were stored under N_2 at −20°C. The stock mixtures were such that when equal volumes were mixed and the two acids separated by argentation TLC, either before or after addition to plasma, 49% of the recovered radioactivity was found in the saturated fatty acid zone (palmitic acid) and 48% in the dienoic acid zone (linoleic acid). For all intravenous injections, 0.1 ml aliquots (4.0 μc) of the two stock solutions were mixed with 0.3 ml of serum obtained from a nonpregnant adult guinea pig (total volume = 0.5 ml).

Half-Lives of Free Palmitic and Linoleic Acids in Maternal Plasma

A pregnant guinea pig was given 0.2 ml of a sodium pentobarbital solution (50 mg/ml) intraperitoneally, then diethyl ether by inhalation to produce anesthesia. The proximal end of a carotid artery was cannulated with polyethylene tubing of I.D. 0.034 inch, O.D. 0.050 inch. A jugular vein was exposed and into it was injected the 0.5 ml mixture of ^{14}C-labeled acids in guinea pig serum. Samples of blood (1.5–1.7 ml) were collected from the carotid artery 30 sec and 2, 4, and 8 min after injection; 0.5 ml of plasma from each sample was used for estimation of radioactivity in free palmitic and linoleic acids. The remaining plasma from the various samples was pooled and used for estimation of the concentration and composition of FFA.

Plasma taken for the determination of radioactivity in free palmitic and linoleic acids was treated as follows. FFA was extracted by the procedure of Dole and Meinertz (7), then isolated by TLC on Silica Gel G. The fatty acids were then eluted from silica gel with diethyl ether and analyzed by argentation TLC. $AgNO_3$ was added to silica gel as follows: a 20 cm × 20 cm × 0.35 mm layer of Silica Gel G on a glass plate was sprayed with 35 ml of a 15% (w/v) solution of $AgNO_3$ in methanol–water 4:1 and heated at 110°C for 45 min (10). The fatty acids were separated on the $AgNO_3$–silica gel with hexane–diethyl ether–formic acid 74:25:1 into saturated, monoenoic, dienoic, and trienoic acid fractions. In one experiment the material in each of the

four fractions was examined by GLC, and the separation of fatty acids into groups according to the degree of saturation was found to be complete. The saturated and dienoic acid zones were scraped from the plate, transferred to vials, and assayed for radioactivity in a Packard Tri-Carb scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.) with 0.5% dimethyl POPOP and 0.03% PPO in toluene as phosphor. Recovery of radioactivity from plasma by this procedure was 76% for palmitic and 67% for linoleic acid. Duplicate samples agreed within 5%.

Placental Transport

A pregnant guinea pig was anesthetized and then tied to a board. The proximal end of a carotid artery was cannulated; a jugular vein was exposed. An incision was made through the anterior abdominal wall, and a horn of the uterus, usually containing a single fetus, was brought to view. The pregnant guinea pig was then placed in a bath of 0.9% saline at 37°C so that the incision was submerged. We cut the uterine wall and then the vitelline and amniotic membranes covering the fetus and delivered the fetus into the bath without pulling or touching the umbilical cord. The lower half of the mother and the entire fetus and umbilical cord were kept under saline throughout the experiment. In this way the fetus was warmed and prevented from breathing air.

A 24-gauge hypodermic needle on a 1 ml syringe, containing a 0.5 ml mixture of ^{14}C-labeled palmitic and linoleic acids in guinea pig serum, was placed in the maternal jugular vein pointing towards the heart. A 20-gauge hypodermic needle on a heparin-coated 5 ml syringe was placed in the umbilical vein of the exposed fetus so that the tip pointed towards the placenta and was almost within it. When suction was exerted as the plunger of this syringe was pulled, the vein collapsed around the needle except at the tip where the walls of the vein were fixed by the placenta; fetal blood emerged from the placenta into the umbilical vein and then through the needle into the syringe. Blood emerging from the placenta could not flow around the needle to the fetus and, therefore, the volume of umbilical vein blood that was collected equaled the total volume of fetal blood coming from the placenta. The volumes of umbilical vein blood collected in our experiments (Table 4, below) during a 1 min interval from fetuses weighing 30, 45, and 60 g (gestational ages 50, 55, and 60 days) were 1.5, 2.1, and 3.5 ml, respectively. These volumes are well within the normal ranges of umbilical venous blood flow (ml/min) as measured by venous occlusion plethysmography (11). Thus, in our experiments the flow rate of fetal blood through the placenta did not appear to be seriously disturbed during the collection period.

Collection of umbilical vein blood was started simultaneously with the injection of ^{14}C-labeled fatty acids into the maternal jugular vein. Maternal blood samples (1.5–1.7 ml) were collected from the carotid artery 30 sec and 2, 4, and 8 min after injection. Maternal and fetal plasma samples were analyzed for radioactivity in free palmitic and linoleic acid. Plasma was obtained from a second fetus in the litter for determination of the concentration and composition of FFA. The concentration and composition of FFA in maternal plasma were also measured.

RESULTS

Concentration and Composition of Plasma FFA

Three groups of animals were examined: pregnant guinea pigs, the fetuses from these animals, and nonpregnant female guinea pigs. Measurements were made on individual fetuses but when it became apparent that the concentrations and compositions of FFA in the plasma of all littermates were essentially the same, blood from littermates was pooled.

The composition of FFA was different in plasma from pregnant animals, from fetuses, and from nonpregnant animals (Table 1). The mole percentage of 18:2 was higher in fetal plasma than in maternal plasma ($P < 0.001$), while the percentages of 16:0 and 18:0 were lower ($P < 0.01$). The percentage of 18:0 was lower in maternal plasma than in plasma from nonpregnant animals ($P < 0.01$), that of 16:0 being higher ($P < 0.02$).

TABLE 1 PLASMA FFA COMPOSITION

	Adult Female, Nonpregnant (n = 6)	Mother (n = 11)	Fetus (n = 9)
		mole % ± SEM	
14:0	1.2 ± 0.2	1.5 ± 0.2	1.4 ± 0.3
16:0	27.8 ± 0.6	31.3 ± 1.0	26.7 ± 1.1
16:1	2.0 ± 0.2	2.6 ± 0.2	2.3 ± 0.1
18:0	18.4 ± 2.1	11.0 ± 0.7	8.0 ± 0.7
18:1	24.7 ± 1.3	25.4 ± 1.4	24.2 ± 1.2
18:2	17.9 ± 1.3	20.4 ± 1.1	28.5 ± 1.4
18:3	8.0 ± 1.2	7.8 ± 0.9	8.9 ± 1.0

Fatty acids are designated by no. of carbon atoms: no. of double bonds.

Blood from adult animals was obtained from the heart or carotid artery. Fetal blood was obtained from the umbilical vein; plasma from littermates was pooled. Measurements were made on maternal and fetal plasma from nine pregnant animals between the 50th and 68th days of gestation (gestation period = 68 days); two additional pregnant animals were examined, but not their fetuses. Pregnant and nonpregnant adult guinea pigs were fed ad libitum or fasted to elevate the concentration of plasma FFA: concentrations were 120–620 μeq/liter in nonpregnant animals, 140–985 μeq/liter in pregnant animals, and 165–960 μeq/liter in fetuses. The composition of FFA did not vary significantly over these concentration ranges, or with fetal age, and the values have therefore been averaged.

The concentration of plasma FFA (Table 2) increases several-fold in pregnant guinea pigs during a 24 or 36 hr fast; it also increases in the fetuses of a fasting mother, though not as much as in the mother. These increases in FFA concentration were not accompanied by changes in composition (Tables 1 and 2).

Comparisons of the concentrations of individual FFA in maternal and fetal plasma (Table 2) indicate that: (*a*) the concentrations of linoleic and linolenic acid are usually the same or higher in fetal plasma than in maternal plasma; (*b*) the concentrations of myristic (see also Table 1), palmitic, palmitoleic, stearic, and oleic acid are higher in maternal plasma than in fetal plasma, except in well-fed animals.

Half-lives of Free Palmitic and Linoleic Acids in Maternal Plasma

The half-lives of free palmitic and linoleic acids, determined from the initial slopes of specific activity–time curves (data shown in Table 3) were found to be 1.3 and 0.7 min, respectively. These half-lives were independent of concentration (Table 3). Constant half-lives result, of course, in constant FFA composition (Table 2). It also follows that with a constant half-life, the amount of a FFA leaving plasma varies exactly and directly with its concentration in plasma.

The rate of disappearance of radioactive free palmitic acid from the maternal circulation is close to being constant from 30 sec to 4 min after injection (Table 3).

TABLE 2 CONCENTRATIONS OF THE MORE ABUNDANT FFA IN MATERNAL AND FETAL PLASMA

	Source of Plasma (M = maternal, F = fetal)																			
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
	Fed				Fasted 12 hr				Fasted 24 hr						Fasted 36 hr					
	μeq/liter																			
16:0	38	35	47	61	141	68	150	70	317	237	288	169	292	207	278	189	298	273		
18:0	17	15	24	26	43	23	52	28	106	64	89	46	95	66	84	66	101	82		
18:1	29	35	28	38	134	65	125	56	259	191	245	176	210	166	272	165	298	254		
18:2	34	61	27	64	64	62	142	86	147	188	202	206	213	231	224	182	177	213		
18:3	15	14	10	16	12	12	71	39	31	33	49	54	97	101	91	71	76	103		
Total (including 14:0 and 16:1)	*140*	165	*140*	210	*410*	240	*560*	285	*890*	740	*910*	680	*960*	800	*980*	700	*985*	960		
Gestational age in days	55		50		62		60		55		64		60		55		62			

Maternal and fetal blood was obtained as described under Table 1. Pregnant animals were fed ad libitum or fasted to elevate the concentration of plasma FFA; they are presented in order of increasing total FFA concentration in maternal plasma (values italicized). Myristic (14:0) and palmitoleic (16:1) acids make up less than 5% of the total plasma FFA (Table 1) and have been omitted from this table.

TABLE 3 HALF-LIVES OF FREE PALMITIC AND LINOLEIC ACIDS IN MATERNAL PLASMA

Animal	Gestational Age of Fetuses	Specific Radioactivities of 16:0 and 18:2 in Maternal Plasma after Injection, Expressed as % of 30 sec Value*						Concentration of FFA in Maternal Plasma		
		2 min		4 min		8 min				
		16:0	18:2	16:0	18:2	16:0	18:2	Total	16:0	18:2
	days	%		%		%		*μeq/liter*		
1†	50	49.2	24.1	15.2	4.9	2.1	0.1	140	47	27
2†	55	41.8	20.8	16.9	11.0	4.6	3.8	980	278	224
3	58	32.2	25.5	13.1	6.1	4.1	1.1	370	137	58
4†	60	39.2	18.8	21.3	10.0	6.6	4.5	960	292	213
5	68	50.2	24.1	21.3	9.5	6.1	4.1	870	256	185
Average		42.5	22.6	17.6	8.3	4.7	2.7			
SEM		4.0	1.0	1.6	1.1	0.8	0.8			

* Palmitic acid-^{14}C and linoleic acid-^{14}C mixed with serum were injected simultaneously into a jugular vein of a pregnant guinea pig; maternal blood samples were collected from a carotid artery 30 sec and 2, 4, and 8 min after injection; the specific radioactivities of free palmitic and linoleic acids in plasma were determined in each sample.

† Data obtained from these animals also appear in Table 4; all the animals are included in Tables 1 and 2.

Theoretical considerations suggest, therefore, that the mixing of injected material with maternal plasma is largely completed by 30 sec.

Placental Transport

Radioactive palmitic and linoleic acids injected into the maternal circulation were found only in the FFA fraction of maternal and fetal plasma, not in phospholipid, triglyceride, or cholesteryl esters, even as long as 2 min after injection. Thus on passing across the placenta from maternal to fetal plasma FFA enter and leave the placental barrier in an unesterified form.

The method used to estimate the rates of transfer of free palmitic and linoleic acids across the placenta in the direction of maternal to fetal plasma was based on the assumption (12) that at any moment the specific radioactivity of a substance being transferred from maternal to fetal plasma is the same as that in maternal plasma. This idea is expressed by the formula mFA-^{14}C/mFA = tFA-^{14}C/tFA, where mFA is the concentration (mμeq/ml) of a fatty acid in maternal plasma, mFA-^{14}C the radioactivity (cpm) of the fatty acid per ml of plasma, tFA and tFA-^{14}C the amount (mμeq) and radioactivity (cpm) of the fatty acid transferred to fetal plasma. If tFA is to be calculated over any period of time, mFA-^{14}C/mFA in the formula must represent the average specific radioactivity of the fatty acid, and tFA-^{14}C the total radioactivity transferred from maternal to fetal plasma during the time interval.

Radioactive palmitic and linoleic acids were injected simultaneously into a jugular vein of an anesthetized pregnant guinea pig. Maternal blood samples were taken from a carotid artery 30 sec and 2, 4, and 8 min after injection; mFA-^{14}C/mFA was estimated at these times. To determine tFA-^{14}C, we collected blood from the umbilical vein of an exposed fetus (all umbilical venous blood was collected for 1 min after the start of the injection of radioisotopes into the mother; during this time no blood passed from the placenta back to the fetus). Decay curves of the specific radioactivities (mFA-^{14}C/mFA) of linoleic and palmitic acid in maternal plasma were constructed from values measured at 30 sec and 2, 4, and 8 min after injection; zero time values were obtained by extrapolation. From these curves the average specific radioactivity of a fatty acid over the 0–1 min interval was estimated graphically. In all cases these averages were found to be within 10–20% of the 30-sec experimental values. Therefore, the 30-sec values for mFA-^{14}C/mFA were used in the formula (Table 4). It must be emphasized that tFA in the formula represents the rate of transfer in one direction (maternal to fetal plasma) and not net transfer, which is the resultant of transfer from maternal to fetal plasma and from fetal to maternal plasma.

TABLE 4 TRANSFER RATES OF FREE PALMITIC AND LINOLEIC ACID FROM MATERNAL TO FETAL PLASMA

		Maternal Plasma (30 sec sample)			Fetal Plasma (0–1 min sample)		
Gestational Age of Fetus	Fatty Acid	Concentration (mFA)	Radioactivity (mFA-^{14}C)	Specific Radioactivity (mFA-^{14}C/mFA)	Radioactivity (tFA-^{14}C)	Transfer Rate*	
days		*mμeq/ml*	*cpm/ml*	*cpm/mμeq*	*cpm*	*mμeq/min*	*mg/24 hr*
50	16:0	47	40,154	854	5,111	6	2.2
	18:2	27	23,540	872	12,650	14.5	5.9
						2.4†	
55	16:0	278	131,826	474	13,058	28	10
	18:2	224	91,226	407	28,918	71	29
						2.5†	
60	16:0	292	77,872	267	9,179	34	13
	18:2	213	47,636	224	15,464	69	28
						2.0†	

Palmitic acid-^{14}C and linoleic acid-^{14}C (5×10^6 cpm in each acid) were injected simultaneously into a jugular vein of a pregnant guinea pig; maternal blood samples were collected from a carotid artery 30 sec and 2, 4, and 8 min after injection (see Table 3). Fetal blood was withdrawn from the umbilical vein of an exposed fetus during the minute following injection of radioactive fatty acids into the maternal circulation; all blood flowing from the placenta was collected. The three pregnant guinea pigs (50th, 55th, and 60th day of gestation) weighed 940, 610, and 735 g and carried four, two, and two fetuses, respectively; the mother of the 50 day old fetus was fed ad libitum, the other two pregnant animals were fasted for 24–36 hr. The volumes of plasma obtained from the 50-, 55-, and 60-day old fetuses were 0.7, 1.0, and 1.7 ml; in all cases plasma volume was approximately one-half of blood volume.

* tFA = (tFA-^{14}C/mFA-^{14}C)mFA.

† Ratio of transfer, 18:2/16:0.

The transfer rates (tFA in mμeq/min) of free palmitic and linoleic acid from maternal to fetal plasma were measured (Table 4) in three pregnant guinea pigs (50th, 55th, and 60th day of gestation). The animal on the 50th day of gestation had been fed ad libitum; the other two animals had been fasted for 24 and 36 hr before the experiment. The transfer rate of linoleic acid was 2.0–2.5 times as great as that of palmitic acid in all three animals. This ratio appeared to be independent of the amounts transferred and of the age of the fetus.

The transfer rates of linoleic and palmitic acid from maternal to fetal plasma were much higher in the fasted animals than in the fed animal. Presumably in fasted animals the transfer of FFA into the fetus is increased, the usual amount of fatty acid is stored in the fetus, and the excess is oxidized (13). In fed animals little or no fatty acid is oxidized in the fetus (14, 15).

The conceptual model upon which our procedure to measure tFA is based probably deviates from the experimental preparation in two ways: it assumes there is no fatty acid pool in the placental barrier to dilute isotope passing across; and it assumes there is no delay in passage. A diluting pool in the placenta would result in an underestimation of tFA; a moderate delay in transit would result in an overestimation. Our measurements of tFA, therefore, must be viewed as tentative until the magnitudes of these effects can be experimentally determined, although there are reasons (as follows) to believe that these effects are small.

Fasting pregnant guinea pigs for 24–36 hr led to a 5- to 7-fold increase in the maternal plasma concentrations of free linoleic and palmitic acids, and a proportional 5- to 6-fold increase in the rates of transfer of free linoleic and palmitic acids from maternal to fetal plasma (Table 4). The fetal plasma concentrations of these free acids increased 3-fold with fasting (Tables 2 and 4). Proportionality between the maternal plasma concentration of an FFA and the calculated rate of transfer (tFA) suggests that any diluting pool either is insignificant or does not change as the plasma concentrations increase. The former possibility is perhaps more likely. The failure of slices of guinea pig placenta to release FFA into a medium containing bovine serum albumin (largely freed of bound fatty acids) also suggests that any FFA pool in the tissue is very small. The placenta in this experiment was perfused with 0.9% saline in situ before it was excised, sliced, and incubated for 3 hr at 37°C under conditions in which fetal adipose tissue released 12 μmoles of FFA into the medium per g of tissue per 3 hr.

Additional confidence in our results can be gained by comparing the rate of transfer (tFA) for linoleic acid with the rate of accumulation (storage). Obviously the true tFA for linoleic acid must equal the rate of accumulation in the fetus plus the rate of oxidation plus the rate of backflow from fetus to mother. There is little or no oxidation of fatty acid in the fetus of a well-fed mother (14, 15). The rate of backflow is unknown but, in any case, tFA must equal or exceed the rate of accumulation. The growth rate (2.5–4.0 g per day) and the fatty acid content (1–1.5% body weight) of 50–60-day old guinea pig fetuses indicate that approximately 5–18 mg of linoleic acid accumulates in a fetus of this age group per day, lesser amounts (5–7 mg) at 50 days, with progressively greater amounts with increasing age. Our measured value for the transfer of linoleic acid from mother to fetus in a well-fed animal on the 50th day of gestation was 5.9 mg (Table 4), which is close to the amount stored in 1 day (5–7 mg) at this age. Thus, if the effect of a diluting pool on the calculation of tFA is small, then any overestimation of tFA by a moderate delay in transit also must be small.

The comparison of transfer per day (5.9 mg) and accumulation per day (5–7 mg) of linoleic acid in the 50-day old fetus (mother well-fed) suggests that the backflow of FFA from fetus to mother is relatively small. If this conclusion is substantiated by further experiments on fed animals, or if backflow of linoleic and palmitic acid occurs in the same ratio as influx, it will be possible to deduce from the ratio of influx into the fetus (approximately 2.0, see Table 4) and from the proportion of linoleic to palmitic acid in fetal tissues (approximately 1.0, see Tables 5–7) that one-half of the palmitic acid stored in the fetus comes from the mother and one-half is synthesized in the fetus.

Lipid Concentration and Total Fatty Acid Composition in Plasma, Adipose Tissue, and Liver

Maternal and fetal tissues were studied at various gestational ages and compared with fed and fasted (24 hr) nonpregnant animals. The analysis of plasma is shown in Table 5. The concentration of lipid in plasma rises in the fetus during the course of normal development, and in the adult with fasting. The composition of the total fatty acid in plasma was found to be similar in mother and fetus and in the fed and fasted nonpregnant animal.

The analysis of adipose tissue is shown in Table 6. In mother and fetus and in the newborn and adult, the composition of total fatty acid in adipose tissue is similar and close to the composition of total fatty acid in plasma.

The analysis of liver is shown in Table 7. The concentration of lipid in liver parallels the concentration in plasma, rising in the fetus during the course of normal development, and in the adult with fasting. The fatty acid composition of the lipid-rich liver of the fetus and of the fasted adult is similar to that of plasma. In the relatively lipid-poor liver of the fed adult there is a higher percentage of stearic and less oleic acid. This

TABLE 5 TOTAL FATTY ACID COMPOSITION AND LIPID CONCENTRATION IN PLASMA

	Mother	Fetus	Mother	Fetus			
	52nd Day of Gestation		64th Day of Gestation		Newborn 4 hr	Adult	Fasted Adult
	mole % of total fatty acids						
14:0	1	1	2	2	1	1	1
16:0	22	22	24	25	22	14	21
16:1	4	3	2	3	2	2	2
18:0	10	10	7	9	10	15	7
18:1	24	19	26	25	21	26	27
18:2	28	34	} 39	29	} 42	} 39	31
18:3	11	13		9			11
20:4					2	3	
	mg/ml plasma						
Concentration of lipid	1.5	2.2	1.2	7	15	1.8	10

TABLE 6 TOTAL FATTY ACID COMPOSITION IN PERIRENAL ADIPOSE TISSUE

	Mother	Fetus	Mother	Fetus		
	57th Day of Gestation		64th Day of Gestation		Newborn 4 hr	Adult
	mole % of total fatty acid					
14:0	2	2	2	2	1	2
16:0	23	26	31	30	26	28
16:1	2	3	3	4	2	3
18:0	6	11	9	13	11	5
18:1	29	24	31	28	26	25
18:2	} 38	} 34	17	16	} 34	28
18:3			7	7		9

may indicate a higher proportion of phospholipid, an important component of membranes (16). The fetal liver cell seems to contain less membrane than the adult liver cell, for even before the concentration of lipid in fetal liver rises above fed-adult values, the fatty acid composition is already like that of plasma (Table 5). Electron microscopic observations and analyses of cell fractions obtained by differential centrifugation have indicated that the fetal liver cell contains approximately $^1/_3$ of the endoplasmic reticulum and mitochondria of the adult liver cell. These structures proliferate after birth. Previous work has shown that there are many biochemical differences between fetal and adult guinea pig liver, important changes taking place in the early postnatal period (17, 18).

When the chemical composition of fetal liver is studied, it should be remembered that the developing liver represents several changing cell populations. In terms of mass, the most important are hepatic cells and extravascular blood-forming cells. Peters (19) has shown that in the liver of the guinea pig fetus, the blood-forming cells represent less than 5% of the total cytoplasmic mass by the 52nd day of gestation (gestational period 68 days). Therefore, biochemical changes observed in studies of excised fetal liver after the 52nd day of the gestational period probably reflect, fairly well, changes in the liver cell.

DISCUSSION

A guinea pig fetus can be made accessible for direct study by delivering it into a warm saline bath through incisions in the mother's abdominal wall and uterus.

TABLE 7 TOTAL FATTY ACID COMPOSITION AND LIPID CONCENTRATION IN LIVER

		Mother (Day of Gestation)		Fetus (Days)			Newborn		
	Adult	57th	64th	52	57	64	4 hr	Fasted Adult	
	mole % of total fatty acid								
14:0	1	1	1	1	1	1	1	2	1
16:0	12	15	18	25	19	25	21	29	25
16:1	1	1	4	2	2	5	3	4	3
18:0	26	22	20	3	8	7	7	5	7
18:1	17	16	17	31	30	29	27	21	25
18:2	} 39	} 44	38	28	} 40	25	} 41	27	30
18:3			3	11		8		12	7
20:4	5	2							
	mg/g liver								
Concentration of lipid	18	45	38	45	90	150	155	120	200

For the fetus, the bath approximates intrauterine conditions. The placenta remains attached to the uterus and, if the umbilical cord is kept entirely submerged in the bath along with the fetus and the lower parts of the mother, the fetus will usually survive in good condition for several hours (11). Apparently the placenta continues to function efficiently in this preparation: the blood in the umbilical vein remains well oxygenated, the electroencephalogram over the frontal cortex of the fetus is normal for at least 15 min (20), and a term fetus (65–68 days of gestation) after 15–30 min in the bath will begin to breathe when lifted out into the air and will survive and grow normally after the umbilical cord is severed. Thus, this preparation should provide a satisfactory system for the study of placental transport.

Evidence for the transport of fatty acids from mother to fetus comes largely from two sources: (*a*) observations of the flow of isotopically labelled fatty acids from the maternal circulation to the fetus; and (*b*) studies comparing the essential fatty acid content of maternal and fetal tissues. The latter studies can give an accurate measure of the net transfer of essential fatty acids from mother to fetus. Isotopic tracers can be used to measure undirectional transfer in a steady state condition. For anatomical reasons, transfer rates from maternal to fetal plasma are much easier to determine than transfer the other way.

Previous work with the guinea pig (3), rabbit (2), and rat (4) had shown that little, if any, isotope-labeled phospholipid, cholesterol, or chylomicron triglyceride enters the fetus from the maternal circulation. Free palmitic acid-^{14}C, however, rapidly passes into the fetus from maternal plasma. Such transfer is much greater in the guinea pig (3) and rabbit (5) than in the rat (21) or sheep (22). This grouping of species emerges again when the concentrations of fatty acid in maternal and and fetal plasma are examined. In the rat (23, 24) and sheep (22, 25), the concentrations of both esterified fatty acid and FFA are higher in maternal plasma than in fetal plasma. In the guinea pig (Tables 2 and 5) and rabbit (5), the opposite condition exists, most dramatically towards the end of gestation.

The essential fatty acid content of fetal tissues also differs between the rat–sheep and guinea pig–rabbit groups. In ruminants (sheep, goat, cow) linoleic and linolenic acid are abundant in the phospholipid and cholesteryl esters of maternal plasma. These fatty acids are scarce, however, in the phospholipid and cholesteryl esters of fetal plasma, in the FFA of maternal plasma, and in the triglyceride of maternal and fetal plasma (26). In the guinea pig, linoleic and linolenic acid make up a large part of the free and esterified fatty acids of both the mother and the fetus (Tables 1, 5–7). Apparently the presence of large amounts of essential fatty acids in fetal tissues is correlated with a high percentage of such acids in the FFA of maternal plasma. This agrees with radioisotopic studies of fatty acid transport which had indicated that free, but not esterified, fatty acids are rapidly passing from the maternal circulation into the fetus (2–4).

A differential rate of placental transfer for essential and nonessential fatty acids was suggested in the guinea pig by differences in the concentration and composition of FFA in maternal and fetal plasma (Tables 1 and 2). Any attempt, however, to relate the composition of FFA in fetal plasma to the maternal–fetal transport of fatty acid must take into account the contribution of fetal adipose tissue to fetal plasma FFA, as well as uptake by fetal tissues and possible backflow from fetus to mother. In a few experiments we found that the composition of the FFA released in vitro by both fetal and maternal adipose tissue was exactly the same as that of maternal plasma. Thus, the enrichment of fetal plasma with free linoleic acid is probably solely the result of placental transport.

To study placental transport in the guinea pig, we chose linoleic and palmitic acids as being representative of the essential and nonessential fatty acids; they are abundant in guinea pig tissues (27) and can be separated easily by chromatography. We found that radioisotopically labeled linoleic and palmitic acids, mixed with serum and injected into the maternal circulation, appear in the fetal circulation in seconds; that they both enter and leave the placental barrier in an unesterified form; and that 2.0–2.5 times more free linoleic acid than free palmitic acid passes from maternal to fetal plasma across the placenta. This ratio appears to be independent of the rates of transfer or fetal age (Table 4). Others have shown that albumin-^{131}I does not cross the guinea pig placenta (28). Therefore on entering the placental barrier an FFA must dissociate from maternal plasma albumin, and on leaving the barrier must associate with albumin in fetal plasma. The tissue between maternal and fetal plasma in the guinea pig placenta consists of two layers of cells separated by a relatively thick basement membrane (29). Thus, any FFA passing across would probably require a protein carrier.

The question arises, why is the rate of linoleic acid transfer (tFA) from maternal to fetal plasma 2.0–2.5 times greater than the rate for palmitic acid when the maternal plasma concentration of free linoleic acid is, on the average, only 0.7 of the free palmitic acid concentration. A simple interpretation (30, 31) of all the information would be that a carrier protein in the placental barrier has a higher affinity for free linoleic acid than for free palmitic acid. However, it is not necessary to postulate existence of such a carrier to explain the *ratio* of transfer rates; the properties of serum albumin appear to be sufficient. Goodman (32) has measured the dissociation con-

stants between several fatty acids and human serum albumin in aqueous solution. If the constants are similar for guinea pig serum albumin, the ratio of the concentrations of *unbound* free linoleic and palmitic acid in maternal guinea pig plasma would be in the range of 2–3. Thus, the ratio of the transfer rates observed for linoleic and palmitic acid (2.0–2.5) could be determined by the relative concentrations of the *unbound* FFA (33, 34) in maternal plasma, or stated equivalently, by the maternal plasma concentrations of the FFA and their relative affinities for serum albumin.

In thinking about the source of fatty acids in the fetus, we have found it helpful to group species into types based on the relative importance of placental transport and endogenous fetal synthesis. The information available for most species is incomplete but a general pattern is discernible, as our discussion has indicated. In the rat, sheep, and probably all domesticated ruminants, the fetus synthesizes most of its own fatty acids; the net transfer of FFA from mother to fetus is relatively low unless the animal is fasted; the fetus contains little linoleic and linolenic acid even though these acids may be abundant in the phospholipid and cholesteryl esters of maternal tissues. Man apparently falls into this group (25).

In the guinea pig and rabbit, the fetus obtains a large part of its fatty acids from the mother; the net transfer of FFA from mother to fetus is high in well-fed animals. The high net flow of FFA from the maternal circulation across the placenta into the umbilical vein results in a large accumulation of esterified fatty acid in fetal liver (35), plasma, and probably adipose tissue. This stored fatty acid is oxidized in the newborn period (14).

Probably types intermediate between the rat–sheep and guinea pig–rabbit groups will be found. Future studies will also have to investigate possible differences between early and late stages of fetal life, as well as the effects of diet and feeding patterns. It is intriguing that in species (guinea pig, rabbit) in which the placental transport of maternal plasma FFA is comparatively large, linoleic acid is a major constituent of maternal plasma FFA.

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