Physiological fatty liver and hyperlipemia in the fetal guinea pig: chemical and ultrastructural characterization

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Abstract During its prolonged period of gestation, the fetal guinea pig gradually develops a striking hyperlipemia (plasma triglycerides ca. 500–1500 mg/dl) and fatty liver (hepatic triglycerides ca. 25% of wet weight). The parenchymal cells of the liver contain not only many fat droplets in the cytoplasm, but also large numbers of osmiophilic particles, interpreted as precursors of plasma lipoproteins, within profiles of the cisternae and secretory vesicles of the Golgi apparatus. Similar particles are found in intercellular spaces, in the space of Disse, and in the hepatic sinusoids. Near the end of gestation, these particles enlarge to the size range characteristic of chylomicrons secreted from the intestinal mucosa after ingestion of fat. At the same time, the hyperlipemia increases and is characterized by the accumulation of particles resembling chylomicrons morphologically and chemically. The results are interpreted as evidence of intense hepatic synthesis and secretion of very low density lipoproteins which may be related to the extensive transplacental transport of free fatty acids known to occur in this species. After birth, the hyperlipemia subsides rapidly and the hepatic steatosis more gradually. The blood plasma of the guinea pig fetus also contains moderate amounts of low density and high density lipoproteins. The latter decrease to barely detectable levels during the first 2 wk of postnatal life. Comparably low levels of high density lipoproteins are found in nonpregnant and pregnant adults.

Supplementary key words free fatty acids · very low density lipoproteins · high density lipoproteins · Golgi apparatus · placental transport · pregnancy

Placental transport of FFA varies widely among mammalian species (1–6). It is particularly extensive in the rabbit and guinea pig (2, 4–6). Fetuses of these two species also store considerable fat in liver (5, 7, 8) and brown adipose tissue (9). The pathways of fat transport within the fetus have received little attention, although it is known that the usual major lipid classes are present in blood plasma. As in adults, plasma FFA removed from the blood of the fetal rabbits reappear shortly in plasma triglycerides (2).

Large species differences exist in the content and composition of plasma lipoprotein classes. Both adult rabbits (10) and guinea pigs (11) have moderate amounts of VLDL and LDL. Adult rabbits, like many other mammals, also have substantial amounts of HDL in plasma (ca. 2 mg/ml) (10). However, in adult guinea pigs HDL cannot be detected by standard ultracentrifugal and electrophoretic procedures (11). Fetuses of these two species differ from other mammals that have been studied in having higher plasma concentrations of cholesterol and triglycerides than their mothers (2, 5, 8).

The guinea pig is a particularly useful animal in which to study developmental phenomena in utero because of the long gestational period (70 days) and the large size and advanced somatic development of the animals at birth (12). The present study was undertaken to extend to the fetus information concerning the unique lipoprotein distribution in the guinea pig and to define sequential changes in lipid content of fetal blood plasma and liver. Fetal plasma was found to contain VLDL, LDL, and HDL at as early as 40 days of gestation. An extraordinary fatty liver and hyperlipemia develop during the last 10 days of gestation and regress rapidly after birth. Since these phenomena are generally considered to represent pathological responses, we have

Abbreviations: FFA, free fatty acids; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins.

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also characterized some of their structural and chemical features.

METHODS

Experimental animals

Pregnant guinea pigs were obtained from a local vendor. Gestational age was determined from mean weight and length within each litter according to Draper (12). Since our fetuses had a higher ratio of length to weight than that given by Draper, we have calculated their age from the mean weights. The pregnant animals were kept for several days at 20°C before the experiment. They had free access to water and food pellets (Berkeley Diet Guinea Pig Food, Foodstuffs Processing Co., San Francisco) which contained 20% protein and 4% fat; this diet was supplemented with lettuce. Fetuses were obtained at Caesarian section and infants at specified times after spontaneous delivery.

Experimental technique

Fluothane was administered by mask, and the level of anesthesia was ascertained from muscular relaxation and reaction to painful stimuli. Fluothane caused little depression of respiration in mothers or fetuses. No local anesthesia was used. The abdominal wall was opened by a midline or ventrolateral incision and the level of anesthesia was then decreased. The uterus was opened and the fetuses were then bled and removed in sequence. For this purpose, a small incision was made through the fetal membranes and the umbilical cord was brought through the incision without permitting the fetus to breathe air. Blood (1.5–5.5 ml) was withdrawn from the umbilical vein through a no. 18 needle under slight suction using a plastic syringe containing sufficient disodium ethylenediaminetetraacetate to make a final concentration of 1–3 mg/ml. The samples were immediately cooled on ice and centrifuged at 4°C for 30 min at 1000 *g*. After obtaining a blood sample, the fetus was decapitated and samples of tissue (approximately 0.3 g) were taken from liver, interscapular brown fat, and perirenal fat. The samples were rapidly weighed, homogenized in 50 ml of ethanol–acetone 1:1 (v/v) in a blender, and the lipids were extracted overnight at 40°C. After all the fetuses had been removed, blood was obtained from the mothers by cardiac puncture through the diaphragm.

Analyses

Lipids were extracted from 1-ml samples of plasma into 5 ml of Dole's mixture (13), and the extract was purified (14). FFA were determined by titration in a two-phase system (13). The extracts containing tissue lipids were filtered and made to a known volume. Lipid phosphorus was determined after digestion in acid (15), and the amount of phospholipids was calculated by assuming a phosphorus content of 4%. Samples for estimation of triglycerides were evaporated to dryness and taken up in chloroform. Silicic acid was added to the chloroform solution in order to remove phospholipids; this procedure was repeated on the supernate. Glyceride-glycerol in the purified extract was assayed (16). Liver glycogen was determined after alkaline digestion (17). Samples of plasma for separation of lipoproteins were pooled within litters or between litters of the same age to obtain 5–6 ml. 10–13 ml of maternal plasma was used for lipoprotein separation. The lipoproteins were separated by preparative ultracentrifugation into VLDL (d < 1.006), LDL (d 1.006–1.063), and HDL (d 1.063–1.21) (10). Each lipoprotein fraction was recentrifuged at the same density for 15–20 hr. LDL and HDL were dialyzed overnight at 4°C against 100 vol of 0.9% NaCl containing 0.04% disodium ethylenediaminetetraacetate. Protein was assayed by the method of Lowry et al. (18), with bovine albumin as standard. The lipids (especially in VLDL) may give rise to turbidity during development of color. The samples and standards were, therefore, extracted twice with 4 vol of diethyl ether (19) before measuring the absorbance at 750 nm. For determination of lipoprotein lipids, samples were extracted overnight in 20 vol of chloroform–methanol 2:1 (v/v). An equal volume of 0.05% sulfuric acid was added and the phases were allowed to separate. The chloroform phase was taken to dryness under nitrogen. The lipids were separated on columns of silicic acid (20), and samples were taken for assay of lipid phosphorus and glyceride-glycerol as described above. Total and free cholesterol were estimated colorimetrically (21) after precipitation as the digitonide (22). Electrophoresis of serum and lipoprotein fractions was performed on agarose gel (23). Analytical ultracentrifugation of low and high density lipoproteins was carried out by standard techniques (24).

Electron microscopy

Small portions of the margin of the left lobe of the liver were cut into small cubes while immersed in 4% osmium tetroxide in distilled water (25). Fixation was continued for 1 hr at 4°C, after which the cubes were rapidly dehydrated in graded concentrations of acetone and embedded in Epon (26). Thin sections were stained with lead citrate (27) and examined in an Hitachi HS-8 electron microscope. Plasma lipoproteins fixed in osmium tetroxide in 0.1 M phosphate buffer (pH 7.3) overnight (28) were examined in a Philips EM 300 electron microscope. Particle diameters were measured

TABLE 1. Concentration of lipids in plasma and liver of fetal and maternal guinea pigs

Gestational Age	Plasma				Liver			
	Free Fatty Acids		Triglycerides		Triglycerides		Phospholipids	
	Fetus[a]	Mother	Fetus[a]	Mother	Fetus[a]	Mother	Fetus[a]	Mother
days	*μmoles/ml*		*mg/ml*		*mg/g*		*mg/g*	
56	0.65	0.35	1.8	0.11				
60	0.72	0.80	6.8	0.75	310	41	23	37
66	0.70	0.60	2.6	0.24	147	18	25	48
68	0.80	1.05	7.5	0.12	230	13	19	32
68	0.80	0.67	16	0.49	254	56	23	37
68	0.74	0.68			142	8.2	25	48

[a] Each value is the mean for that litter.

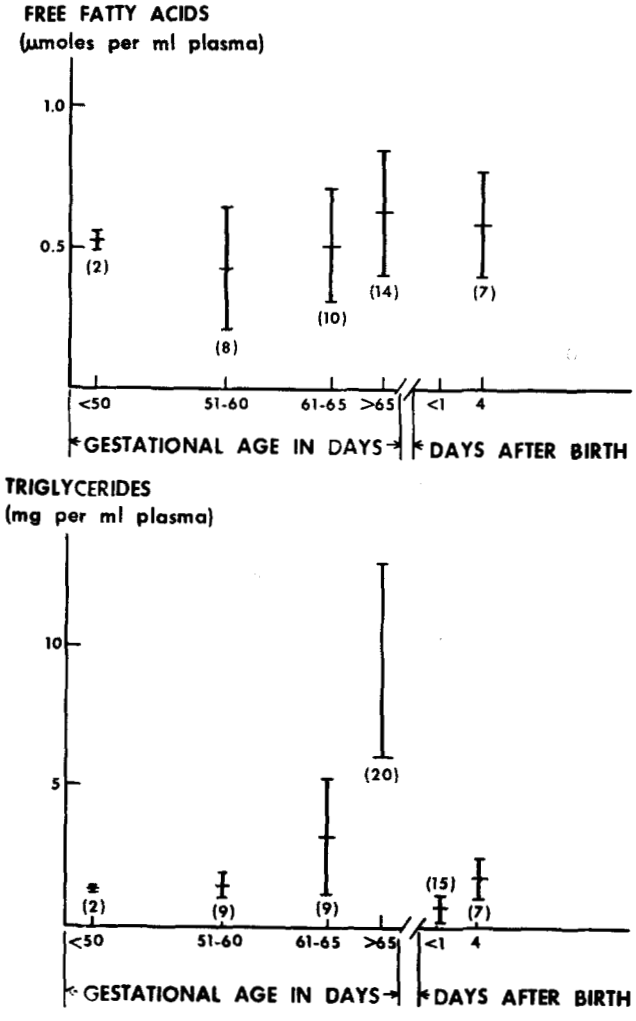

FIG. 1. Changes in free fatty acids and triglycerides of blood plasma during pre- and postnatal development of guinea pigs. Mean values, SD, and number of observations are given (for triglycerides at > 65 days, only the SD above the mean is shown).

directly on the photographic plates using a magnifier containing an accurate scale. Magnification was calibrated using a replica of a diffraction grating with 54,864 lines/inch.

RESULTS

Chemical changes in the liver and blood plasma during gestation and after birth

The concentration of FFA was about 0.5 mM in both fetal and maternal plasma and did not change significantly during gestation (Fig. 1 and Table 1). Triglyceride levels were higher in fetal than in maternal plasma even during the earlier part of gestation (< 50 days) and increased enormously during the last 10 days. In some litters the plasma was creamy, and lactescent particles layered on the surface of the serum upon storage at 3°C. The mean triglyceride level near term was 5.5 mg/ml of plasma. The distribution of these values was skewed and ranged from 2 to 20 mg/ml. After birth, the triglyceride level fell rapidly. Fig. 2 shows that the liver of young fetuses (50 days) also had a high triglyceride content (7% of wet weight) and that this level increased in parallel with that of the plasma up to about 25% of wet weight during the last 10 days of gestation. After birth, the content of triglyceride in liver fell more slowly than that in plasma. Concentrations of triglycerides in maternal plasma and liver were much lower than in the fetus and did not change systematically during the latter part of gestation (Table 1). Concentration of phospholipids in fetal liver was lower than in maternal liver, but increased when content of triglyceride fell 4 days after birth. Glycogen in fetal liver increased rapidly during the last week of gestation. The glycogen level in the newborn was even higher than in late-gestational fetuses.

Ultrastructural features of fetal hepatocytes

An hepatocyte from a fetus of approximately 52 days gestation is shown in Fig. 3. Numerous profiles of mitochondria of diverse shapes are the most prominent feature of this micrograph. Closely following the contours of most mitochondria are cisternae of the rough-surfaced endoplasmic reticulum. Smooth-surfaced endoplasmic reticulum organized as in adult livers is seem-

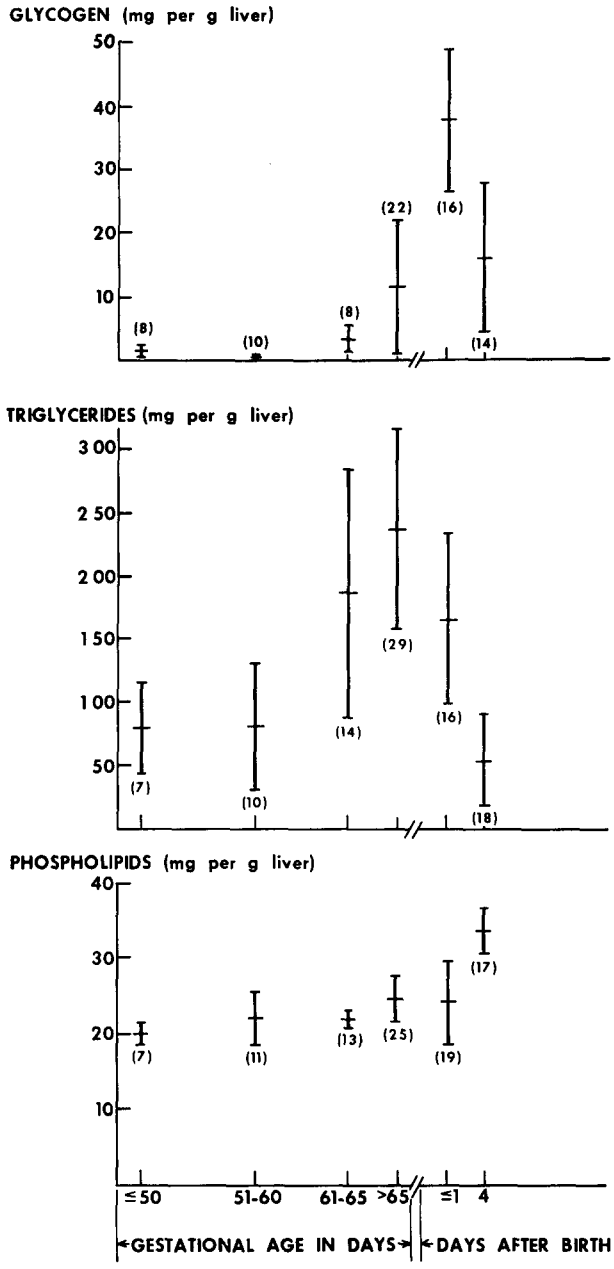

FIG. 2. Changes in hepatic glycogen, triglycerides, and phospholipids during pre- and postnatal development of guinea pigs. Mean values, SD, and number of observations are given.

ingly absent, although a few smooth-surfaced vesicles can be observed. The Golgi complex is well represented in livers of this age. This organelle is composed of a few short cisternal profiles together with a mass of small vesicles and some larger vacuoles which contain numerous small particles (Fig. 5). In the rat, such particles have been shown to be lipoproteins with chemical and physical properties similar to those of plasma VLDL (29). Bile canaliculi are rudimentary and the vasculature is not well defined. Large numbers of erythrocyte precursors are found in livers of this age. Small portions of these cells can be observed at the upper and lower right-hand margins of Fig. 3.

Liver cells from fetuses near term were strikingly different from those just described. In the cell shown in Fig. 4, large droplets of lipid crowd the cytoplasm. These droplets are not bounded by a typical trilaminar membrane; rather, a thin electron-dense line is observed at the surface. In addition, elements of the endoplasmic reticulum may be associated with the surface of these droplets. The mitochondria do not exhibit any remarkable change when compared with the previous state. A greater abundance of endoplasmic reticulum is seen in hepatocytes of this stage of gestation. In addition, numerous free ribosomes are found, many of which are associated in characteristic linear and spiral arrays. The Golgi complex has enlarged considerably, the most conspicuous change being the formation of numerous large vacuoles which contain a large number of lipoprotein particles (Fig. 6). More significantly, the lipoprotein particles are now much larger than those seen earlier. Glycogen is represented in tissues by this method as finely granular areas from which other organelles are excluded.

Extracellular lipoprotein particles are commonly observed in livers from animals late in gestation (Figs. 4, 7, and 8). In Fig. 7, the extracellular space between adjacent hepatocytes is filled with a large number of round profiles which resemble those seen in the Golgi complex. In addition, an image is seen in this figure which is interpreted as representing fusion of the vacuole membrane with the cell membrane, resulting in the release of the contained lipoprotein particles into the extracellular space. Similar particles are observed in the sinusoidal lumen (Fig. 8). The mean diameter of the extracellular lipoprotein particles in this liver, as measured in thin sections, was 2160 Å; that of particles within vacuoles of the Golgi complex was 2198 Å.

Lipoproteins in fetal and maternal blood plasma

Electrophoresis of samples from fetal plasma suggested the presence of four classes of lipoproteins. A large amount of stainable material of $d < 1.006$ remained in the trough; the bulk of the remainder migrated to the pre-β region with some trailing back to the trough (Fig. 9, top). Two other bands of $d > 1.006$ were present in the β and α regions (Fig. 9, bottom). These observations suggested the presence of the usual classes of lipoproteins. A more detailed study of ultracentrifugally separated lipoprotein classes was, therefore, undertaken. Table 2 shows that the fetus has all the usual major ultracentrifugal classes of lipoproteins. There was very little lipoprotein at $d > 1.21$. In two samples obtained near term, phospholipid concentrations in this

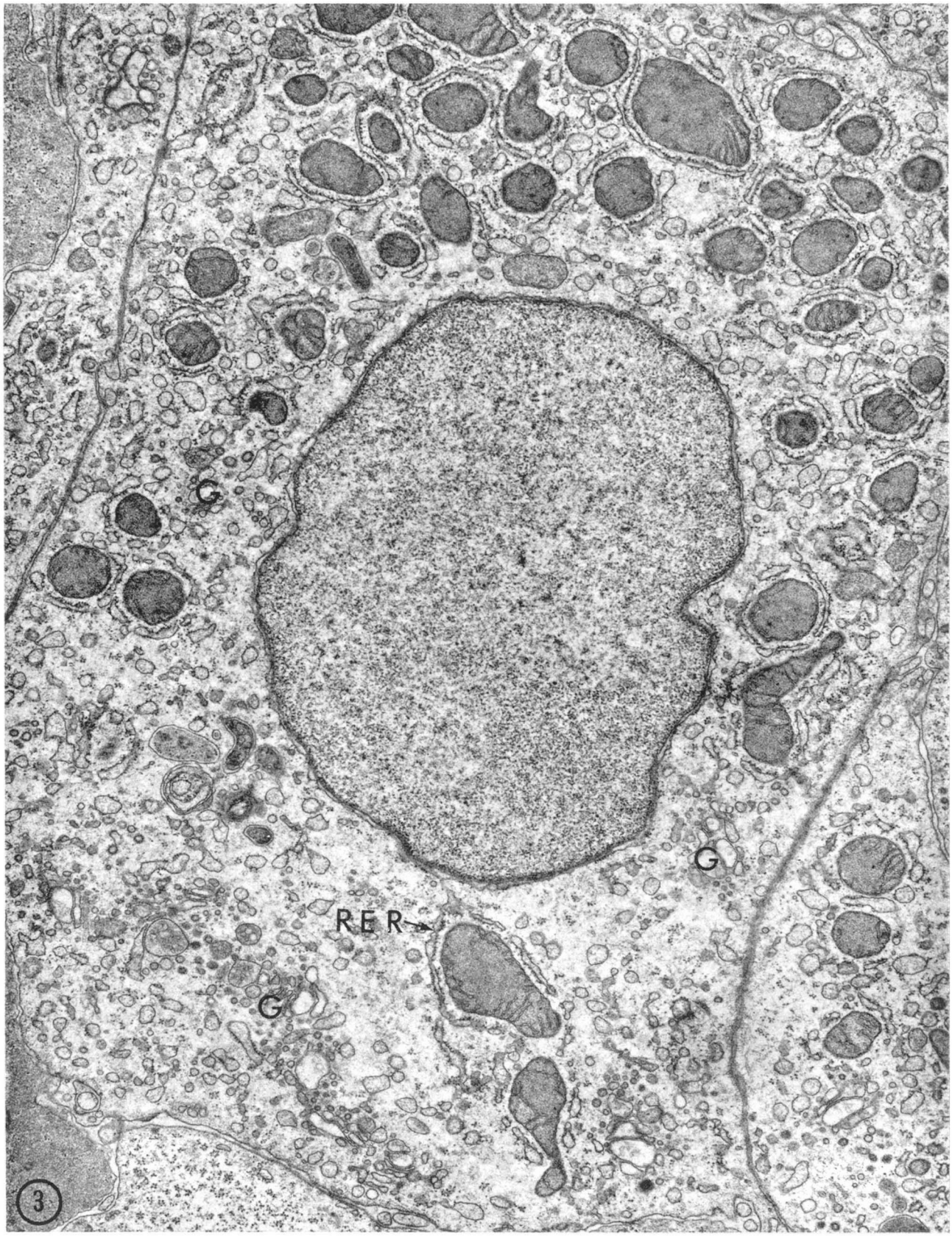

FIG. 3. Portions of several hepatocytes from a guinea pig of approximately 52 days gestational age. The nucleus of one cell occupies the center of the field. Numerous mitochondria are present, many of which are partially surrounded by cisternae of the rough-surfaced endoplasmic reticulum (*RER*). The Golgi complex (*G*) is seen at several places in this micrograph. Bile canaliculi are not observed, and the normal adult vascular relationships are not developed as yet. ×15,000.

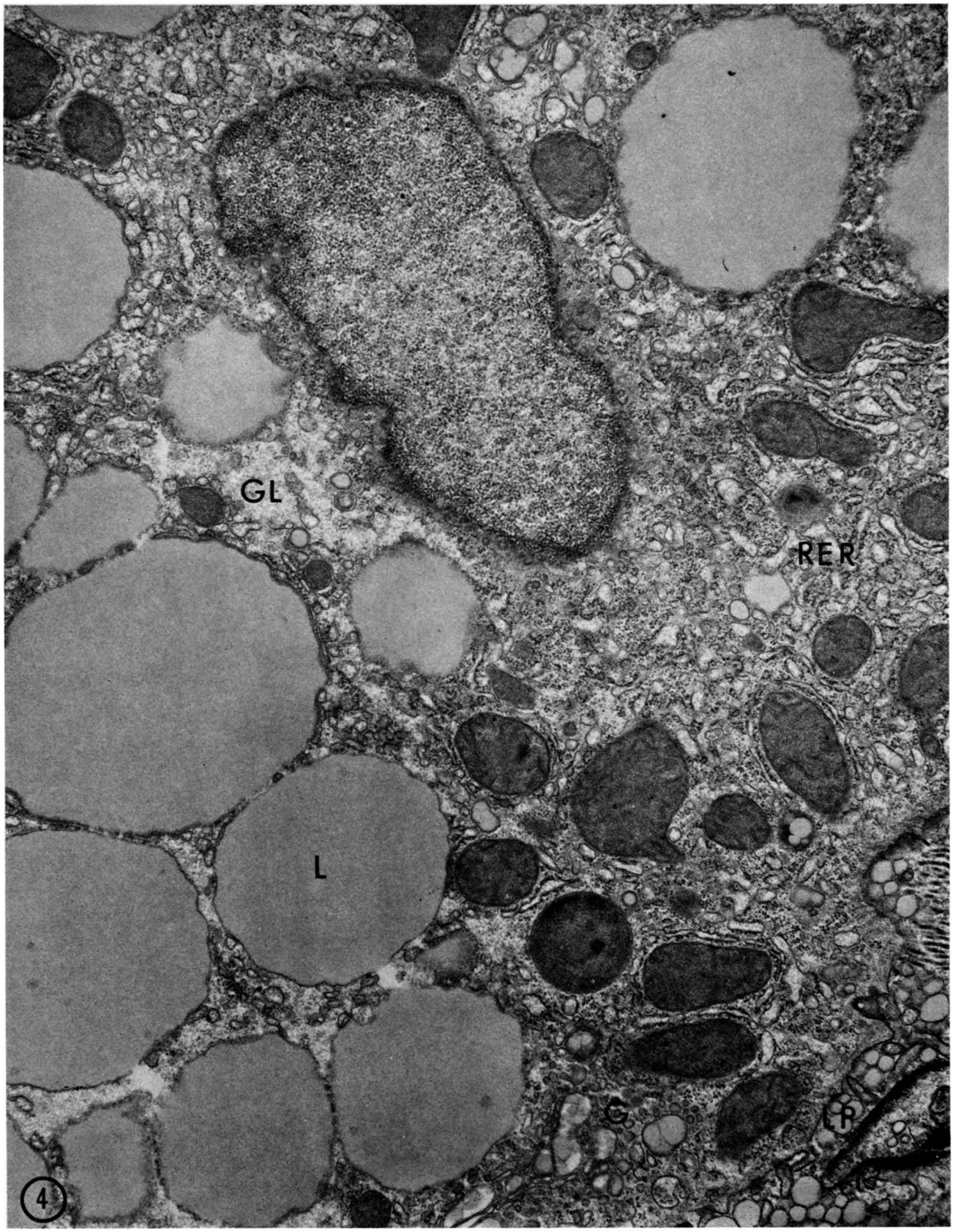

FIG. 4. Part of an hepatocyte from a fetal guinea pig of approximately 68 days gestational age is seen in this micrograph. Numerous large lipid inclusions (*L*) are apparent. The rough-surfaced endoplasmic reticulum (*RER*) is more abundant at this time than at the earlier stage. The electron-lucent areas represent areas of glycogen storage (*GL*). A Golgi complex (*G*) is seen near the lower margin of the picture. Numerous lipoprotein particles are seen in the extracellular space (*LP*). ×15,000.

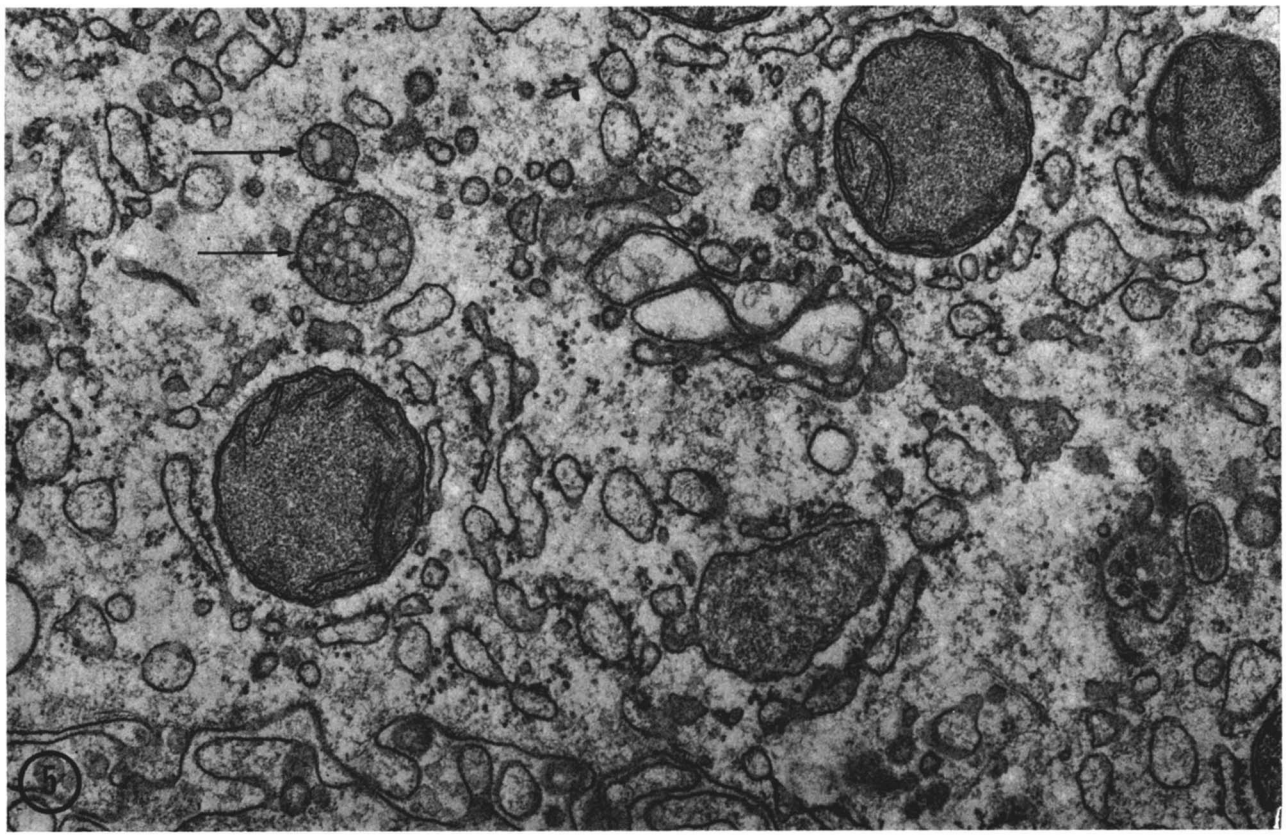

FIG. 5. Golgi complex in an hepatocyte of a 52-day-old guinea pig fetus. The lipoprotein particles are small and of relatively uniform size. Two secretory vesicles are seen at the arrows. ×35,000.

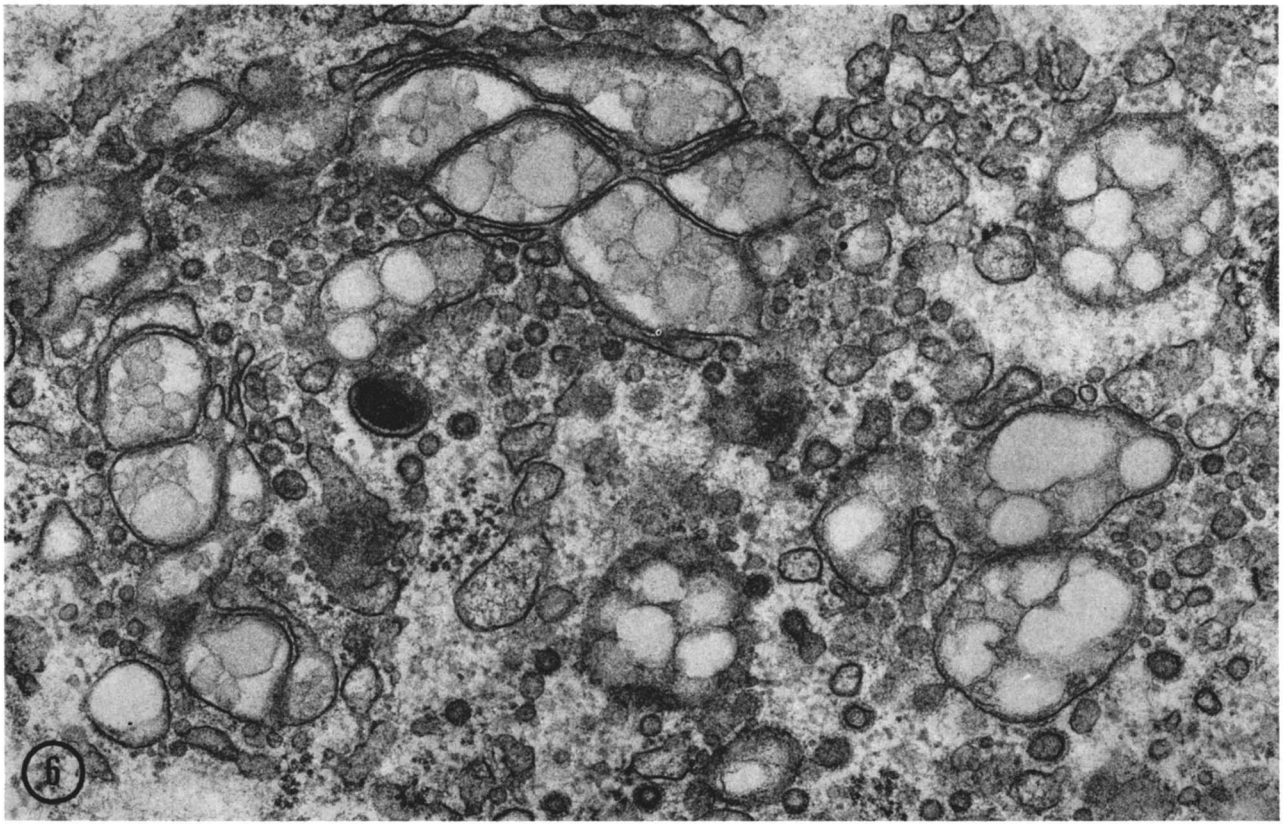

FIG. 6. Golgi complex in an hepatocyte of a 68-day-old guinea pig fetus. The Golgi complex is more extensive, and the contained lipoprotein particles are larger and more heterogeneous in size. ×35,000.

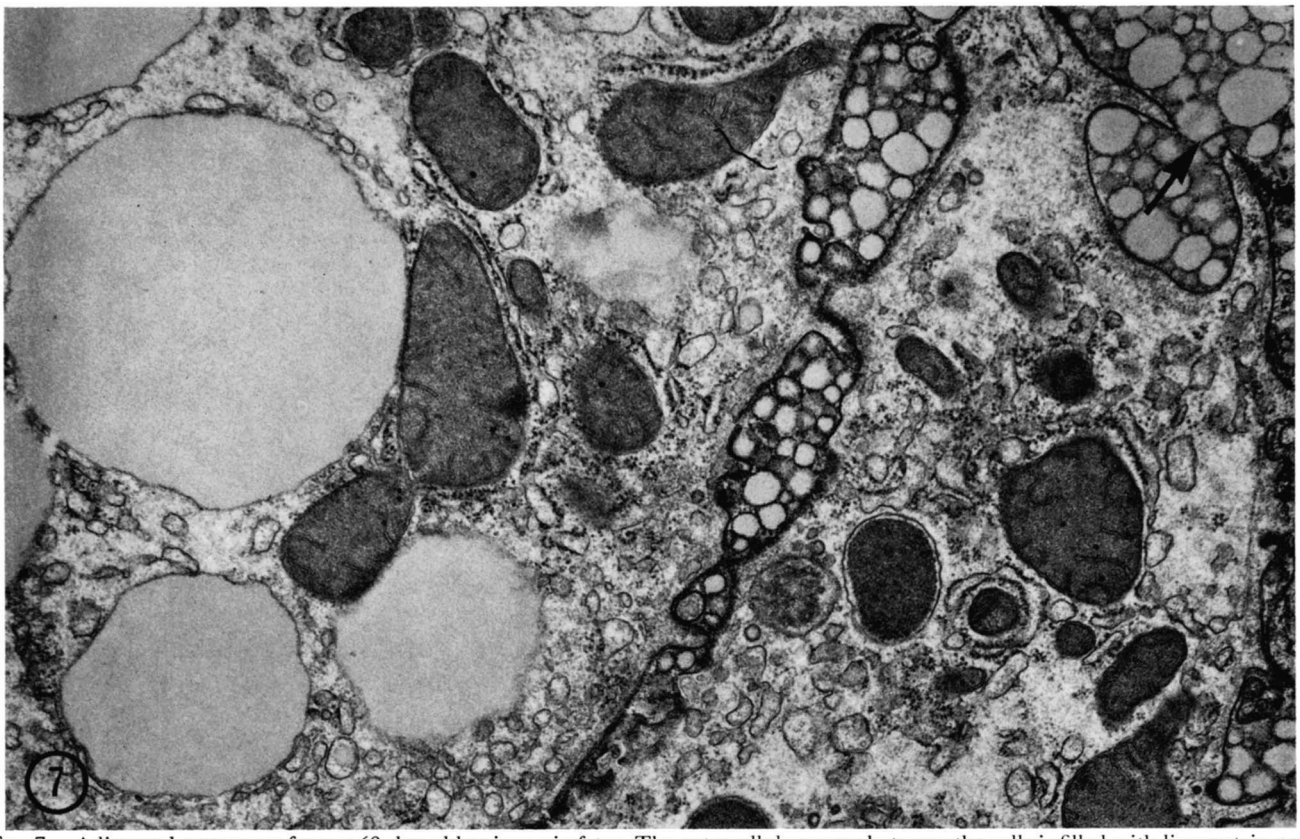

FIG. 7. Adjacent hepatocytes from a 68-day-old guinea pig fetus. The extracellular space between the cells is filled with lipoprotein particles. At the arrow, one can observe an image which suggests the emptying of a secretory vesicle into the extracellular space. ×16,500.

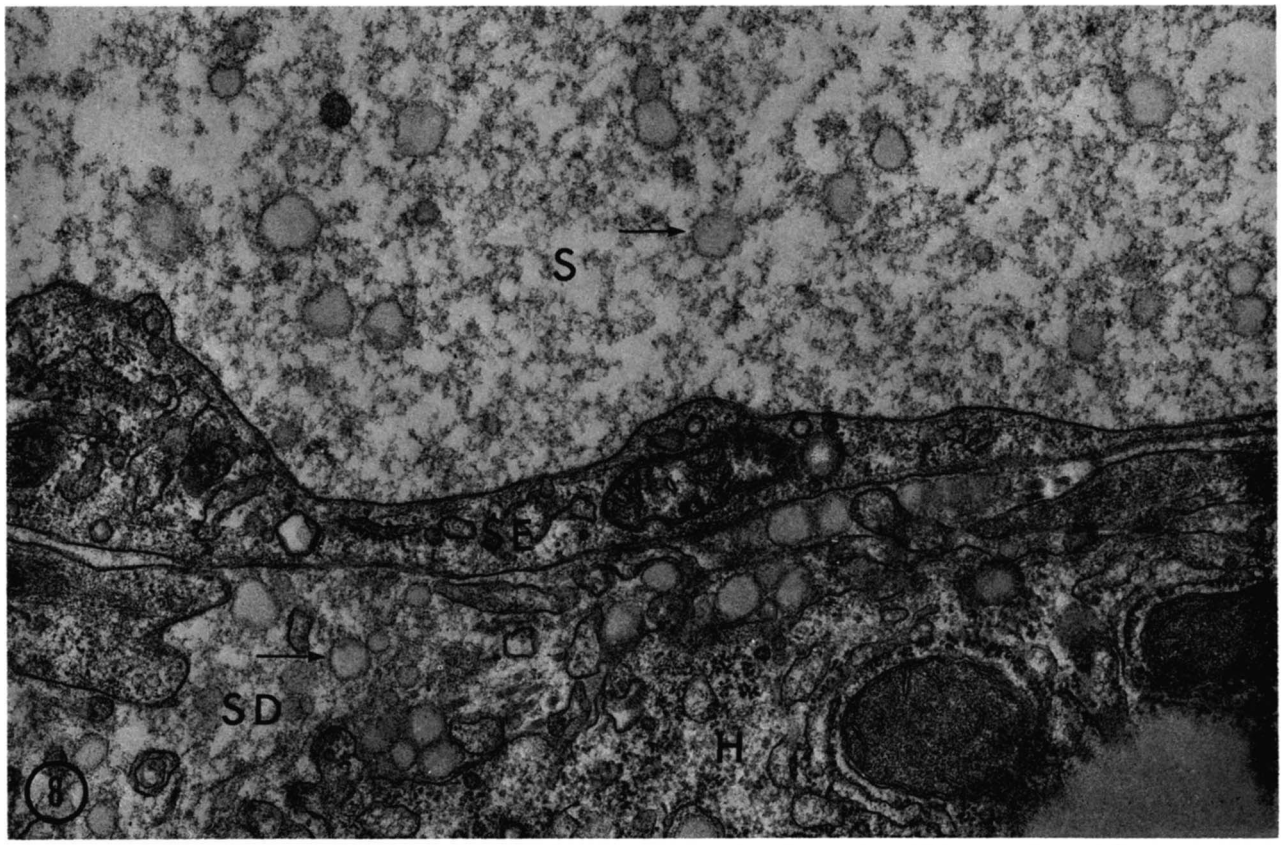

FIG. 8. Portion of an hepatocyte (*H*) from a 68-day-old guinea pig fetus, showing also the sinusoidal endothelium (*SE*) and the sinusoidal lumen (*S*). Lipoprotein particles (arrows) are seen in the space of Disse (*SD*) and in the sinusoidal lumen. ×29,500.

TABLE 2. Plasma lipoproteins in the fetal guinea pig[a]

Density	Pool No. and Estimated Age	Proteins		Phospholipids		Triglycerides		Cholesteryl Esters and Free Cholesterol		Esterified Cholestero
	days	*μg/ml*	*wt %*	*μg/ml*	*wt %*	*μg/ml*	*wt %*	*μg/ml*	*wt %*	*%*
<1.006	A (67)	615	3.5	955	5.4	15600	87.5	651	3.8	9
	B (60)	171	3.5	206	4.2	4400	90.2	102	2.1	18
	C (60)	61	3.0	113	5.6	1800	89.5	41	2.0	16
	D (62)	353	2.8	955	7.5	11150	86.7	380	3.0	<10
1.006–1.063	A	101	37.2	35	13.0	60	22.4	72	27.0	78
	B	90	32.7	30	10.9	41	15.0	115	41.6	81
	C	66	25.4	32	12.4	22	8.6	139	53.6	91
	D	314	24.2	275	26.5	126	9.0	567	40.0	84
1.063–1.21	A	175	59.0	53	18.0	38	12.8	30	10.2	78
	B	297	51.0	118	20.3	38	6.5	128	22.2	83
	C	187	44.6	101	24.6	12	2.8	121	28.6	86
	D	329	46.5	168	23.8	70	9.9	139	19.6	87

[a] Values are expressed as concentrations in unfractionated plasma.

TABLE 3. Plasma lipoproteins in the pregnant guinea pig[a]

Density	Animal No. and Estimated Age of Litter	Proteins		Phospholipids		Triglycerides		Cholesteryl Esters and Free Cholesterol		Esterified Cholesterol
	days	*μg/ml*	*wt %*	*μg/ml*	*wt %*	*μg/ml*	*wt %*	*μg/ml*	*wt %*	*%*
<1.006	A (60)	13	9.0	168	11.2	114	76	5.7	3.8	8.7
	B (65)	65	13.5	94	19.0	293	58	42.7	8.8	16.5
1.006–1.063	A	119	34.2	57	16.3	6.9	1.7	204	50	92
	B	106	28.2	136	36.6	13.0	3.7	117	31	68
1.063–1.21	A	24[b]		3.2		3.8		4.1		
	B	24[b]		16		5		5		

[a] Values are expressed as concentrations in unfractionated plasma.
[b] Probable contamination with nonlipoprotein protein (see text); percentage composition was, therefore, not calculated.

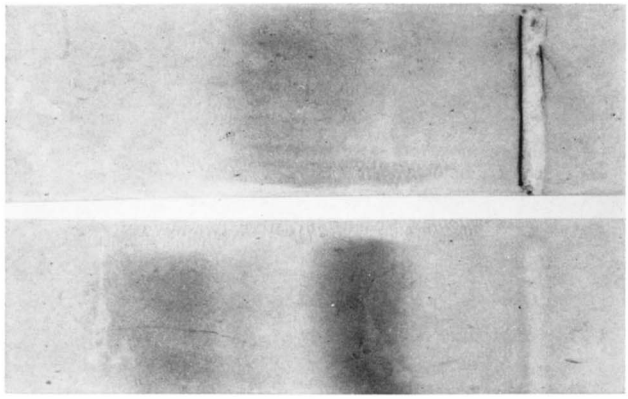

FIG. 9. Electrophoresis of fetal plasma after ultracentrifugal separation into fractions of d < 1.006 (top) and > 1.006 (bottom). Both fractions were concentrated fourfold over serum, and 50 μl was applied to the trough.

fraction were 5 and 15 μg/ml of plasma, and the concentration of cholesterol was less than 10 μg/ml. The VLDL migrated from the trough to the pre-β region, the LDL had β mobility, and the HDL had α-1 mobility. None of these fractions contained detectable material with differing mobility. The VLDL had a very high content of triglycerides, a low content of proteins, and a ratio of triglycerides to phospholipids near 20:1. The "surface" material (proteins, phospholipids, and free cholesterol) constituted about 11% of the total weight. This chemical composition is very similar to the composition usually found in chylomicrons.[1] The low content of surface material suggests that these VLDL particles are unusually large. The degree of esterification of cholesterol in the VLDL was much lower than has previously been observed in other species under physiological conditions. While the absolute level of the VLDL from the fetus varied 10-fold, the chemical composition was almost constant. More than 98% of the triglycerides in fetal plasma were in VLDL; therefore, changes in triglyceride levels reflect precisely changes in the level of the VLDL in the fetus. The maternal animals had a lower absolute level of VLDL (Table 3). Their VLDL had a higher content of protein and a lower content of triglyceride than the fetal VLDL, indicating a more typical distribution of particle size. The surface material of the maternal VLDL constituted 23 and 40% of

[1] A sample of chylomicrons obtained from intestinal lymph of a fat-fed adult guinea pig by gel filtration (30) had the following composition: protein, 2.1%; phospholipids, 4.5%; free cholesterol, 0.8%; cholesteryl esters, < 0.1%; triglycerides, 92.6%.

total weight in the two animals studied. As in the fetus, very little of the cholesterol was esterified. LDL was present at similar levels in fetal and maternal animals. HDL was present in much higher amount in the fetus than in the mother, where it could just be detected (see also Table 4). To exclude the presence of an HDL of unusual density, analysis for cholesterol was performed on the protein-rich material sedimenting at d > 1.21. Less than 10 μg/ml of plasma was found. In addition, concentration of phospholipid was very low in the d > 1.21 fraction. In three animals near term, values were 3, 6, and 9 μg/ml, similar to those observed in the fetus.[2]

To confirm the suggested dimensions of the VLDL in the fetus, osmium-fixed samples were also examined by electron microscopy. The distribution of the diameters of the particles was asymmetrical, the mean diameter being about 850 Å, with 6–8% having diameters above 1500 Å (Fig. 10). The larger particles, however, contributed more than half of the total volume, consistent with the chemical composition of the VLDL as shown in Table 1.[3] In the electron micrographs, fetal VLDL particles frequently adhered to each other (even when the fixation was done at 20°C). Such adhesion is more common for particles of the size of chylomicrons than for VLDL of usual size.[4]

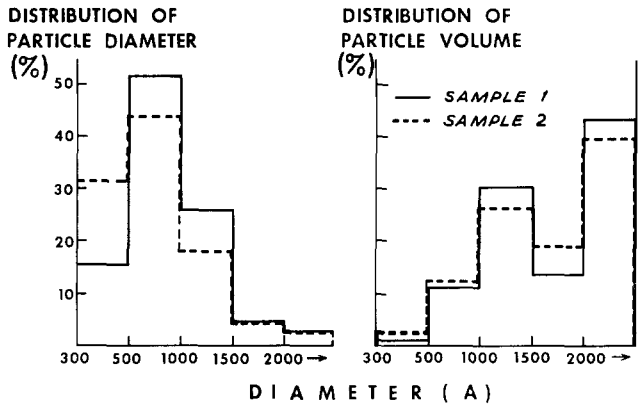

FIG. 10. Dimensions of VLDL obtained from two pools (A and B in Table 2) of guinea pig plasma late in gestation. The diagram shows the distribution of particles according to diameter (left), and the contribution of particles in each range of diameter to total volume (right), assuming that the particles are spheres. 600 particles were counted from each sample.

[2] By thin-layer chromatography, material with R_F values similar to those of lecithin, sphingomyelin, and lysolecithin was detected in this fraction.

[3] Similar measurements of d < 1.006 lipoproteins from intestinal lymph of a fat-fed adult guinea pig gave similar results; mean diameter was 724 Å, with 3% of the particles having diameters above 1500 Å.

[4] Jones, A. L. Personal communication.

It can be seen from Tables 2 and 3 that the level of HDL is much higher in the fetus than in the adult animal. The level was still high 4 days after birth (Table 4) and fell during the next 2 wk. Because the extent of contamination of HDL by other serum proteins can be expected to be constant in absolute terms, the reduction of the HDL protein levels in the newborn is probably greater than shown in Table 4. In several states, there is an inverse relationship between the levels of VLDL and HDL (31), but in fetal guinea pigs, levels of both VLDL and HDL were higher than in adults. Levels of VLDL fell rapidly after birth (Fig. 1), while HDL decreased slowly. Analytical ultracentrifugal analysis of pooled serum from three fetal guinea pig littermates showed only about 10 mg/dl of resolvable VLDL ($S_f°$ 20–400), 49 mg/dl of LDL with peak $S_f°$ value of 7, and 45 mg/dl of HDL with $F_{1.20}$ values between 1 and 14. These values for LDL and HDL are consistent with the chemical concentrations of their constituents shown in Table 2. The low values for VLDL with $S_f° < 400$ provide further documentation of their unusually large size. In the serum of the mother of this litter, no VLDL or HDL could be resolved; LDL concentration was 40 mg/dl with a peak $S_f°$ of 2.2.

TABLE 4. Concentration of high density lipoprotein constituents in infant and nonpregnant adult guinea pigs[a]

Days after Birth	No. of Animals	Proteins	Phospholipids	Cholesterol
		μg/ml	μg/ml	μg/ml
1	5	252 ± 81	120 ± 35	59 ± 27
4	3	235 ± 121	102 ± 53	37 ± 31
17	5	90[b] ± 28	4.2 ± 1.6	<5
(Adult)	4	62[b] ± 29	9.5 ± 6.1	<5

[a] Values are expressed as concentrations in unfractionated plasma (means ± SD).

[b] Probable contamination with nonlipoprotein protein.

DISCUSSION

In the fetal guinea pig, the facile transplacental transport of FFA (4–6) is accompanied by high levels of VLDL. A similar phenomenon exists in rabbits (2, 8). These associations raise the possibility that transport of lipids in VLDL in fetal mammals is related to the influx of FFA. Hepatic secretion of VLDL in adult mammals is a function of uptake of FFA into the liver (32). The ultrastructural appearance of hepatocytes in the guinea pig fetus suggests rapid synthesis of VLDL precursors and their secretion into the extracellular space. Although the plasma VLDL in the fetus resemble chylomicrons in dimensions and chemical composition, it seems unlikely that the intestine of the fetus partici-

pates substantially in the formation of triglyceride-rich lipoprotein, since absorption of fat in the intestine should be limited to that derived from endogenous sources. In subsequent studies, we have found that the triglyceride content of the small intestine is low and does not increase during the period of increasing plasma levels of triglycerides.[5] Increased dimensions of lipoprotein particles in the Golgi apparatus of hepatocytes and of putative hepatogenous VLDL in plasma have been observed in other states associated with increased synthesis and secretion (33). This phenomenon, which permits increased quantities of triglycerides to be transported with relatively little increase in synthesis o "surface" constituents of the lipoprotein complex (protein, phospholipids, and unesterified cholesterol), reaches extreme proportions in the guinea pig fetus and emphasizes the difficulty of distinguishing exogenous and endogenous triglyceride-rich lipoproteins solely on the basis of particle dimensions and associated physical and chemical properties. The reason for extracellular VLDL measured from thin sections of liver having apparently larger diameters than those in plasma is not clear. It could represent individual variation. Alternatively, smaller particles may not be distinguished readily in thin sections. It is also possible that the sinusoidal membrane acts as a selective sieve (34) and that larger particles enter the plasma compartment less readily from the space of Disse than do smaller ones. This possibility is consistent with the apparent tight packing of particles in the intercellular spaces (Fig. 7).

The triglyceride content of fetal guinea pig liver is extraordinarily high, as observed previously by Flexner and Flexner (7) and Hershfield and Nemeth (5). The triglycerides are present not only in "nascent" VLDL but also in fat droplets lying free in the cytosol. This fatty liver is not the result of disease, but rather appears to represent the normal response to high influx of FFA transported across the placenta. A similar phenomenon may exist in the rabbit (8). Electron micrographs of thin sections of fetal mouse liver appear to show VLDL precursors in the Golgi apparatus, although in this species they are much more prominent on the first postnatal day (35). Additional studies have shown that FFA are indeed the source of hepatic triglycerides in the guinea pig fetus.[5] The fetal liver contains about 1 g of triglyceride at term (2% of body weight), an amount approximating triglyceride stores in brown adipose tissue. The rapid fall in hepatic and plasma triglycerides after birth suggests that hepatic triglycerides, like those of brown fat, are readily available for energy needs. The present studies do not define the mode of transport of lipid into adipose tissue, but it is attractive to postulate that the mechanisms by which plasma triglycerides are taken up from the blood into adipose tissue in adults (36) also obtain in the fetus.

The mechanism of the increasing hyperlipemia during the last 10 days of gestation is uncertain. The associated changes in the liver and the accompanying storage of fat in adipose tissue suggest that increased transport of triglycerides participates in this phenomenon. However, we have no information about the efficiency of removal mechanisms in the fetus. The rapid fall in plasma triglycerides after birth does not help to elucidate the role of clearing efficiency, since the rate of formation of VLDL in liver may fall when placental transport of FFA ceases at birth. The reduction of plasma triglycerides precedes that in the liver, in keeping with the presumed direction of flow.

The presence of low but easily detectable quantities of HDL in the guinea pig fetus indicates that their virtual absence in the adult does not have solely a genetic basis. The extremely low levels of HDL in the adult guinea pig are associated with markedly reduced ability of the plasma to promote interaction of fat emulsions with lipoprotein lipase, the key enzyme of triglyceride transport (37). However, the influence of the low levels of HDL on removal of triglycerides from the blood remains to be established.

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[5] Bøhmer, T., and R. J. Havel. Unpublished data.

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