Microsomal and lysosomal enzymes of triacylglycerol metabolism in rat placenta

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The placenta plays a major role in transporting lipid to the developing foetus. Since previous studies have suggested that placental lipid transport involves intermediate esterification steps, we investigated selected microsomal and lysosomal enzymes of triacylglycerol metabolism in rat placenta. Between gestational days 10 and 14, microsomal phosphatidic acid phosphatase specific activity was 6-fold greater than the activity in adult rat liver. Phosphatidic acid phosphatase activity decreased 50% on day 15. Studies employing several different phosphorylated substrates indicated a high degree of substrate specificity. Lysosomal triacylglycerol lipase and cholesterol esterase activities decreased about 50% between days 15 and 18, then rose late in gestation. No changes were observed in the specific activities of fatty acid:CoA ligase, glycerolphosphate acyltransferase, lysophosphatidate acyltransferase, diacylglycerol acyltransferase or diacylglycerol cholinephosphotransferase during the final 12 days of gestation. Kinetic observations (competitive inhibition by alternative substrates, pH-dependence and thermal inactivation) were consistent with the hypothesis that glycerol phosphate and dihydroxyacetone phosphate can be acylated by a single microsomal enzyme in placenta. Except for fatty acid:CoA ligase, the activities of microsomal and lysosomal enzymes of triacylglycerol metabolism were comparable with those in adult rat liver. These observations are consistent with physiological studies suggesting that triacylglycerol synthetic and degradative pathways are very active in rat placenta.

In non-ruminant mammals, the placenta functions to transfer maternally-derived fatty acids to the foetus (Hull & Elphick, 1979; Hahn, 1982). Approx. 50% of rat foetal fatty acid is derived from the mother (Hummel *et al.*, 1974, 1975, 1976; Hahn, 1982). Whether maternal non-esterified fatty acids or acylglycerols carried by maternal lipoproteins are the source of transported fatty acids has not been established. The molecular steps involved in the transport process are also unknown. Fatty acids may be derived from serum triacylglycerol by action of placental lipoprotein lipase (Hull & Elphick, 1979) or from receptor-mediated uptake of serum lipoproteins and subsequent hydrolysis of triacylglycerol and cholesteryl esters by lysosomal acid lipase (Winkel *et al.*, 1980). Human placental slices incubated with [³H]-

palmitate synthesized triacylglycerol at a rate independent of the amount of glucose in the incubation medium (Szabo *et al.*, 1973a). Triacylglycerol synthesis was impaired by the addition of fluoride, suggesting a requirement of glycerol-3-P produced during glycolysis (Szabo *et al.*, 1973b). Since the placenta contains little triacylglycerol and since calculated turnover times of rat placental triacylglycerols and phospholipids were 7.6 and 23 h respectively (Hahn, 1982), fatty acid transfer across the placenta may involve intermediate esterification steps to form glycerolipids. The glycerolipids might subsequently be secreted directly into the foetal circulation or fatty acids might be released to the foetus after placental intracellular hydrolysis (Hahn, 1982; Szabo *et al.*, 1973a,b).

Few of the relevant enzymes of glycerolipid metabolism have been examined in placenta. Synthesis of triacylglycerol from long-chain fatty acids occurs by a series of enzymes whose active sites, in liver, face the cytosolic surface of the endoplasmic reticulum (Bell *et al.*, 1981; Bell & Coleman, 1980).

Abbreviations used: glycerol-3-P, *sn*-glycerol 3-phosphate; DHAP, dihydroxyacetone phosphate; LDL, low-density lipoprotein.

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Long-chain fatty acids are activated by fatty acid:CoA ligase (acyl-CoA synthetase; EC 6.2.1.3) to form acyl-CoA thioesters. Glycerol-3-*P* is then sequentially acylated by *sn*-glycerol-3-phosphate acyltransferase (EC 2.3.1.15) and lysophosphatidate acyltransferase to form phosphatidate. A possible alternative route involves the acylation of DHAP by DHAP acyltransferase (EC 2.3.1.42) and the subsequent reduction of acyl-DHAP to form lysophosphatidate. It has been suggested that the glycerol-3-*P* acyltransferase and DHAP acyltransferase in rat tissues are dual functions of a single microsomal enzyme (Schlossman & Bell, 1976, 1977; Coleman & Haynes, 1983a). Action of phosphatidate phosphatase (EC 3.1.3.4) forms diacylglycerol, which can be acylated in the *sn*-3 position by diacylglycerol acyltransferase (EC 2.3.1.20) or allowed to react with CDP-choline or CDP-ethanolamine by action of cholinephosphotransferase (EC 2.7.8.2) or ethanolaminephosphotransferase (EC 2.7.8.1) to form phosphatidylcholine or phosphatidylethanolamine respectively. Intracellular hydrolysis of triacylglycerol is catalysed by one or more triacylglycerol lipases (EC 3.1.1.3). The lysosomal triacylglycerol lipase from human placenta has been purified (Burton & Mueller, 1980), but other intracellular placental triacylglycerol lipases have not been investigated. Apart from rat cholinephosphotransferase (Chepenik *et al.*, 1977; Karp *et al.*, 1973), and human lysosomal acid lipase (Burton & Mueller, 1980), these enzymes of glycerolipid synthesis and degradation have not been studied in placenta.

Since the enzymes of glycerolipid metabolism may play a key role in placental lipid transport and since alterations in activity occur in other placental enzymes during gestation (Diamant & Shafir, 1972), we examined the activities of six microsomal enzymes of glycerolipid synthesis as well as lysosomal triacylglycerol lipase in rat placenta during the final 12 days of the 22 days of gestation.

Experimental procedures

Materials

DL- α -Glycerophosphate, β -glycerophosphate, mannose 6-phosphate, Triton X-100, egg lecithin phosphatidic acid, *N*-ethylmaleimide, bovine serum albumin (essentially fatty acid-free), 5,5'-dithiobis-(2-nitrobenzoic acid), dihydroxyacetone, the dimethyl ketal of DHAP and Dowex 50 were purchased from Sigma. 1-Oleoylglycerol 3-phosphate and 1,2-*sn*-dioleoylglycerol were from Serdary. Palmitoyl-CoA, ATP and CoA were from P-L Biochemicals. Sodium dodecyl sulphate was from BDH Biochemicals. Carrier-free [32 P]P_i, Aquasol, CDP-[14 C]choline and [3 H]palmitic acid

were from New England Nuclear. [3 H]Palmitoyl-CoA (Merrill *et al.*, 1982), [32 P]DHAP (Schlossman & Bell, 1976), and [3 H]glycerol-3-*P* (Schlossman & Bell, 1978) were synthesized by previously reported methods. Glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglycerate kinase, glycerol kinase and 3-phosphoglycerate were from Boehringer Mannheim. Materials for measurement of alkaline phosphatase were obtained from Smith-Kline Instruments, Inc. (Spin Chem).

Methods

Timed pregnant Charles River CD rats were allowed free access to Purina rat chow. Between 08:00h and 09:00h on selected days before expected birth (days 10–22 of gestation), a pregnant rat was decapitated and the placentas were rapidly removed. Litter size varied from 10 to 14 fetuses. For each determination, placentas from an entire foetal litter were combined. Placentas were minced and homogenized with five up-and-down strokes in a motor-driven Teflon/glass homogenizer at moderate speed in ice-cold medium 1 [0.25M-sucrose/1 mM-EDTA/10 mM-Tris/HCl (pH 7.4)].

The homogenate was centrifuged at 1000g for 10 min and the pellet was resuspended in medium 1, and centrifuged at 1000g for 10 min. The resulting pellet was discarded. The combined supernatants were centrifuged at 25000g for 10 min. The resulting pellet was termed lysosomal. The supernatant was centrifuged at 100000g for 1 h to obtain a microsomal fraction. Portions were resuspended in medium 1 and stored at -20°C . Once thawed for assay, each preparation was discarded. Total particulate preparations from rat liver were obtained by centrifuging the homogenate at 100000g for 1 h. Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Enzyme assays

Fatty acid:CoA ligase activity was measured using 50 μM -[3 H]palmitate and 5 mM-ATP as described previously (Banis & Tove, 1974). Glycerol-3-*P* acyltransferase activity was assayed using 75 μM -palmitoyl-CoA and 300 μM -[3 H]glycerol-3-*P*, and DHAP acyltransferase activity was measured using 75 μM -palmitoyl-CoA and 1.5 mM-[32 P]-DHAP (Schlossman & Bell, 1976). Lysophosphatidate acyltransferase activity was determined spectrophotometrically (Lands & Hart, 1965) using 55 μM -oleoylglycerol 3-phosphate and 50 μM -oleoyl-CoA. Phosphatidate phosphatase activity was determined by monitoring P_i release (Mavis *et al.*, 1978) using 1.0 mM-phosphatidate. To exclude the possibility that hydrolysis of glycerol-3-*P* produced by deacylation of phosphatidate was contributing to the phosphatase measurement (Sturton &

Brindley, 1978), 300 μM - $[^3\text{H}]$ glycerol-3-*P* was incubated in the presence of 1.0 mM-phosphatidate under conditions identical with those that were employed to measure phosphatidate phosphatase activity. The water-soluble products were extracted (Schlossman & Bell, 1976) and chromatographed on Whatman 1 paper in 1.0 M-ammonium acetate (pH 7.5)/100% ethanol (7:13, v/v). No $[^3\text{H}]$ glycerol-3-*P* was hydrolysed under those conditions. Diacylglycerol acyltransferase activity was assayed using 30 μM - $[^3\text{H}]$ palmitoyl-CoA and 200 μM -1,2-*sn*-diacylglycerol dispersed in acetone (Coleman & Bell, 1976). Greater than 95% of the product was triacylglycerol by t.l.c. on silica gel G in heptane/di-isopropyl ether/acetic acid (15:10:1, by vol.) (Breckenridge & Kuksis, 1968). Cholinephosphotransferase activity was measured using 100 μM -CDP- $[^{14}\text{C}]$ choline and 100 μM -1,2-*sn*-diacylglycerol dispersed in acetone (Coleman & Bell, 1977). Lysosomal triacylglycerol lipase activity was measured at pH 5.5 using 250 μM - $[^3\text{H}]$ tri-oleoylglycerol and lysosomal cholesterol esterase activity was measured at pH 5.5 using 250 μM -cholesteryl $[1-^{14}\text{C}]$ oleate (Coleman & Haynes, 1983b). Alkaline phosphatase was measured spectrophotometrically at pH 10.5 using 15 mM-*p*-nitrophenyl phosphate (McComb & Bowens, 1972).

Assays were performed at 23°C except for phosphatidate phosphatase, alkaline phosphatase, triacylglycerol lipase and cholesterol esterase, which were performed at 37°C. All assays were proportional to the time and the amount of protein employed. The substrate concentrations employed gave maximal activities.

Inhibition by N-ethylmaleimide

Total particulate or microsomal fractions (1.0 mg of protein/ml) were incubated with *N*-ethylmaleimide (1.0 mM) for 15 min at 4°C and portions were removed for immediate assay of glycerol-3-*P* acyltransferase and DHAP acyltransferase activities.

Results

Phosphatidate phosphatase

Phosphatidate phosphatase activity varied markedly during the final 12 days of gestation (Fig. 1). Between days 10 and 14, phosphatidate phosphatase activity was about 57 nmol/min per mg of microsomal protein. The activity decreased on day 15 and remained at that level through the remainder of gestation. In contrast, adult rat liver phosphatidate phosphatase measured by identical methods was about 9 nmol/min per mg of microsomal protein (Table 1). To ensure that the measurements in placental microsomal fractions

did not erroneously result from variable recovery of subcellular fractions, activities were investigated in total particulate fractions from placenta and in supernatants obtained after centrifugation at 100000g for 1 h. Specific activities from total placental homogenate decreased 2-fold between days 14 and 16, similar to the decrease observed in microsomes, indicating that the microsomal specific activities were an accurate reflection of changes occurring during gestation. Activities remained low throughout the remainder of the gestational period. Soluble phosphatidic acid phosphatase activity was 12.6 ± 2.1 nmol/min per mg of protein and did not change during the last 12 days of gestation.

To test the possibility that the phosphatase assay was not specific for phosphatidic acid, other phosphorylated compounds were incubated under identical conditions with microsomes obtained from day-12 and day-22 placentas. The effect of MgCl_2 was also examined, since Mg^{2+} has been variably reported to increase or decrease phosphatidic acid phosphatase activity (Bell & Coleman, 1980). With 1.0 mM-phosphatidic acid, the specific activities were 78.2 and 23.0 nmol/min per mg of protein on days 12 and 22 respectively. MgCl_2 at 5 mM inhibited phosphatidic acid phosphatase 70 and 66% in microsomes from day-12 and day-22 placentas, respectively, but had no effect on phosphatase activities that were measured by using alternative substrates. On day 12, specific activities obtained by using 1 mM-3-phosphoglycerate, 1 mM-*sn*-glycerol 2-phosphate, 2 mM-DL-*sn*-glycerol 3-phosphate, or 5 mM-mannose 6-phosphate were 15–100-fold lower than those observed when phosphatidic acid was used. No difference was noted in the activities from day-12 to day-22 microsomes with the alternative phosphorylated substrates. Thus the activity appeared to be a specific phosphatase activity.

The placental isoenzyme of human (Galski *et al.*, 1981) and rat (Moriyama *et al.*, 1976) alkaline phosphatase rises during pregnancy. To determine whether the phosphatidic acid phosphatase activity was, in part, a function of placental alkaline phosphatase, microsomes were incubated under conditions employed to measure human serum alkaline phosphatase, using the fluorescent substrate *p*-nitrophenyl phosphate. Rat placental alkaline phosphatase specific activity from day-12 microsomes was 45 nmol/min per mg of protein. Activity increased to 400 nmol/min per mg of protein by day 22, in contrast with phosphatidic acid phosphatase activity, which decreased during this time period. Phosphatidic acid phosphatase activity at pH 10.2, which is optimal for alkaline phosphatase, was 20% of the activity measured at pH 7.0. MgCl_2 at 5 mM did not affect alkaline phos-

Fig. 1. Time course showing changes in specific activity of phosphatidic acid phosphatase in rat placental microsomes. Data are reported as means $\pm$ S.D. for three to six separate litters. Points without error bars indicate a single experiment.

phatase activity. Rat placental alkaline phosphatase activity, like human alkaline phosphatase (Kottel & Fishman, 1981), was inhibited 100% by 10mM-phenylalanine; the phosphatidic acid phosphatase activity was unaffected by phenylalanine. These data clearly distinguish phosphatidic acid phosphatase and alkaline phosphatase activities from placenta.

Lysosomal triacylglycerol lipase and cholesterol esterase activities

Rat placenta lysosomal triacylglycerol lipase and cholesterol esterase specific activities were similar to those observed in adult rat liver (Table 1 and Fig. 2). Both activities appeared to decrease between gestational days 14 and 19, but the range of activity varied considerably (Fig. 2). Evidence has been presented to support the hypothesis that the two lysosomal activities in placenta are functions of a single enzyme (Burton & Mueller, 1980). Changes observed during the final 12 days of gestation are consistent with this hypothesis.

Microsomal glycerol phosphate acyltransferase and DHAP acyltransferase activities

Rat tissues contain mitochondrial and microsomal glycerol phosphate acyltransferase activities and peroxisomal and microsomal DHAP acyltransferase activities (Bell & Coleman, 1980). Microsomal glycerol phosphate and DHAP acyltransferase activities are inhibited more than 90% by incubation with *N*-ethylmaleimide, whereas non-microsomal activities are resistant to *N*-ethylmaleimide inhibition (Bell & Coleman, 1980). When placental total particulate preparations were incubated with *N*-ethylmaleimide, 15% of the glycerol phosphate acyltransferase activity was resistant to inhibition, indicating that 85% of the glycerol phosphate activity in rat placenta is microsomal.

Data from several rat tissues (Schlossman & Bell, 1976, 1977; Coleman & Haynes, 1983a) and from differentiating 3T3-L1 adipocytes (Coleman & Bell, 1980) strongly suggest that microsomal gly-

Table 1. Specific activities of selected enzymes of triacylglycerol and phosphatidylcholine metabolism in rat placenta and adult rat liver

Placenta values are means $\pm$ S.D. for 30–43 litters of pregnant rats between 10 to 22 days of gestation, except for phosphatidic acid phosphatase, triacylglycerol lipase, and cholesterol esterase, results for which are derived from three litters from day 22†. Unless otherwise indicated, activities were measured in microsomal subcellular fractions. Adult liver values are means $\pm$ S.D. from five separate adult 250–300g male and female rats§.

Enzyme	Specific activity (nmol/min per mg)	
	Organ	Adult liver§
Fatty-acid-CoA ligase	...	37.0 $\pm$ 3.5
Glycerol phosphate acyltransferase	Placenta	1.52 $\pm$ 0.41
Lysophosphatidic acid acyltransferase	...	35.1 $\pm$ 9.5
Phosphatidic acid phosphatase	Placenta	9.3 $\pm$ 1.4
Diacylglycerol acyltransferase	...	5.3 $\pm$ 0.6
Diacylglycerol cholinephosphotransferase	Placenta	8.0 $\pm$ 1.1
Acid triacylglycerol lipase†	...	7.55 $\pm$ 2.41
Acid cholesterol esterase†	Placenta	1.59 $\pm$ 0.45

* *N*-Ethylmaleimide-resistant activity was <10%.

† Activity measured in lysosomal subcellular fraction.

‡ Mean result $\pm$ S.D. from day-22 placenta.

§ Some of these values have been reported previously (Coleman & Haynes, 1983a).

Fig. 2. Time course showing changes in specific activity of acid triacylglycerol lipase (○) and acid cholesteryl esterase (●) in rat placenta lysosomes

Data are reported as means $\pm$ S.D. for three to six separate litters.

cerol phosphate and DHAP acyltransferases are dual functions of a single enzyme. This hypothesis was tested in microsomes from rat placenta on gestational days 19 and 20. Glycerol phosphate and DHAP acyltransferase activities had identical pH-dependences, with a pH optimum of 7.5–8.0. A second DHAP acyltransferase optimum was observed at pH 5.0. The pH 5.0 activity was 20% of that seen at pH 8.0 and probably represents peroxisomal contamination of the microsomal preparation (Bell & Coleman, 1980). When microsomal fractions were heated and samples were removed at various times for assay of the glycerol phosphate and DHAP acyltransferase activities at 23°C, the time courses of heat-inactivation were identical for the two activities.

Glycerol phosphatase acyltransferase activity had a $V_{\max}$ of 3.05 nmol/min per mg of protein and an apparent K_m for glycerol phosphate of 36 μ M, similar to that observed in rat brain, intestinal mucosa, lung, and kidney (Schlossman & Bell, 1977). DHAP inhibition of glycerol phosphate acyltransferase was tested with 0.375 and 1.0 mM-DHAP. DHAP appeared to inhibit competitively with an apparent K_i that ranged between 0.34 and 0.49 mM. DHAP acyltransferase activity had a $V_{\max}$ of 1.96 nmol/min per mg of protein and an apparent K_m of 0.90 mM, similar to that reported from foetal-rat liver (Coleman & Haynes, 1983a). Glycerol phosphate tested at 0.2 and 0.35 mM appeared to be a competitive inhibitor of the

DHAP acyltransferase activity, with a K_i that ranged between 0.48 and 0.54 mM.

Other microsomal enzymes of acylglycerol synthesis

During gestational days 10 up and to and including 22 no significant changes occurred in the specific activities of fatty-acid-CoA ligase, glycerol phosphate acyltransferase, lysophosphatidic acid acyltransferase, diacylglycerol acyltransferase, or diacylglycerol cholinephosphotransferase and thus the values for separate days are not reported. Table 1 shows the specific activities of these enzymes in placenta microsomes during the final 12 days of gestation and compares them with activities in microsomes from adult rat liver. Fatty-acid-CoA ligase specific activity was about 20-fold lower in rat placenta than in adult rat liver, but is comparable with the activity reported from kidney, brain and skeletal muscle (Polokoff & Bell, 1977). Glycerol phosphate acyltransferase specific activity was similar to that reported from adult rat liver (Table 1) and other rat organs (Schlossman & Bell, 1977), except adipocytes, in which the specific activity was 3-fold higher (Schlossman & Bell, 1976). Lysophosphatidic acid acyltransferase specific activity was 3-fold lower in placenta than in liver. Diacylglycerol acyltransferase, the enzyme unique to triacylglycerol synthesis, had a specific activity similar to that in adult rat liver (Table 1), but was about 12-fold lower than the activity reported from isolated fat-cells (Coleman & Bell, 1976). Without added dioleoylglycerol, the diacylglycerol acyltransferase activity was less than 2.0% of the activity measured when 200 μ M-dioleoylglycerol was present, suggesting that little endogenous diacylglycerol is present in the microsomal fractions.

Diacylglycerol cholinephosphotransferase specific activity in microsomes from rat placenta was similar to the activity from adult rat liver and did not vary during late gestation. The activity in the absence of added dioleoylglycerol was 6.0% of that measured when 100 μ M-dioleoylglycerol was present, consistent with a previous report (Chepenik *et al.*, 1977) concluding that little endogenous diacylglycerol is present.

Discussion

It has been suggested that placental transfer of fatty acid to the foetus requires transitional esterification steps to form triacylglycerol and phospholipids. Although variation in fatty acid transfer during gestation has not been studied in rat placenta, we measured specific activities during the final 12 gestational days because the amount of fatty acid transferred to the foetus appears to increase during gestation in the rabbit and the guinea

pig (Hull & Elphick, 1979). No changes were noted in the specific activities of five of the microsomal enzymes of triacylglycerol synthesis; however, normal placental growth during this time would provide a large increase in total activity (placental weights increased from 0.07 g to 0.42 g between gestational days 13 and 21). The specific activities of five of the microsomal enzymes of glycerolipid synthesis were 35% or more of the activity observed in adult rat liver, an organ that plays a major role in triacylglycerol metabolism. The fatty acid-CoA ligase activity, however, was comparatively low, similar to the activity measured in tissues such as skeletal muscle, kidney, brain and lung (Polokoff & Bell, 1977). Both diacylglycerol acyltransferase and diacylglycerol cholinephosphotransferase specific activities in placenta microsomes were similar to those measured in adult rat liver. Although a comparison of specific activities may not reflect the capacity of a tissue to synthesize triacylglycerol, the relatively high activities of diacylglycerol acyltransferase and diacylglycerol cholinephosphotransferase are compatible with the hypothesis that transient esterification occurs during placental lipid transport.

Several studies have suggested that microsomal glycerol phosphate and DHAP acyltransferase activities in rat organs (Schlossman & Bell, 1976, 1977; Coleman & Haynes, 1983a) and in 3T3-L1 adipocytes (Coleman & Bell, 1980) are dual functions of a single enzyme. In placenta the patterns of reciprocal competitive inhibition, pH-dependence and thermolability are similar to those reported for other rat tissues and support the hypothesis that acylation of glycerol phosphate and DHAP occurs at a single active site.

Lysosomal cholesteryl esterase and triacylglycerol lipase might be expected to play a role in placental transport of fatty acids if the transport depends, in part, on the uptake of serum lipoproteins or on the hydrolysis of newly synthesized triacylglycerol, as proposed by several investigators (Hahn, 1982; Szabo *et al.*, 1973a). Human placental trophoblastic cells in primary culture can take up LDL by receptor-mediated endocytosis (Winkel *et al.*, 1980). It is believed that lysosomal cholesterol esterase releases fatty acids and cholesterol from LDL cholesterol esters for steroid-hormone biosynthesis (Winkel *et al.*, 1980). Lysosomal lipases may also degrade intracellular triacylglycerol storage droplets (Mooney & Lane, 1981). The specific activity of lysosomal acid lipase in placenta is comparable with the activity observed in adult rat liver and could function to hydrolyse internalized triacylglycerol from lipoproteins or endogenously synthesized triacylglycerol.

In 10–14-day placenta microsomes, phosphatidic acid phosphatase activity was 6-fold higher

than that observed in adult rat liver (Table 1). Even at the end of gestation, placenta phosphatidic acid phosphatase specific activity was 2.8-fold higher than the activity measured in adult rat liver microsomes (Table 1) and was similar to that reported from 3T3-L1 adipocytes (Coleman *et al.*, 1978). Adipocyte and liver phosphatidic acid phosphatase activity changes in response to diet and hormonal treatment and may play a regulatory role in triacylglycerol biosynthesis (Lamb & Fallon, 1974; Brindley & Sturton, 1982; Bell & Coleman, 1983). In differentiating 3T3-L1 adipocytes (Coleman *et al.*, 1978) and in perinatal rat liver (Coleman & Haynes, 1983a), however, phosphatidic acid phosphatase activity increased the least of the triacylglycerol synthetic activities. Further studies are needed to clarify the role of phosphatidic acid phosphatase in placental triacylglycerol and phosphatidylcholine biosynthesis.

These investigations provide the first developmental study of the microsomal enzymes of triacylglycerol and phosphatidylcholine biosynthesis and the lysosomal triacylglycerol lipase and cholesteryl esterase from rat placenta. Of particular interest is the decrease during gestation of a single enzyme activity of the synthetic pathway while the remaining activities do not change. These studies provide an initial descriptive characterization upon which future investigations on placental lipid metabolism and lipid transport can build.

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References

- Banis, R. J. & Tove, S. B. (1974) *Biochim. Biophys. Acta* **348**, 210–220
- Bell, R. M. & Coleman, R. A. (1980) *Annu. Rev. Biochem.* **49**, 459–487
- Bell, R. M. & Coleman, R. A. (1983) *Enzymes 3rd Ed.*, in the press
- Bell, R. M., Ballas, L. M. & Coleman, R. A. (1981) *J. Lipid Res.* **22**, 391–403
- Breckenridge, W. C. & Kuksis, A. (1968) *Lipids* **3**, 291
- Brindley, D. N. & Sturton, R. G. (1982) in *Phospholipids* (Hawthorne, J. N. & Ansell, G. B., eds.), pp. 179–213, Elsevier Medical Press, Amsterdam
- Burton, B. K. & Mueller, H. W. (1980) *Biochim. Biophys. Acta* **618**, 449–460
- Chepenick, K. P., Borst, D. E. & Waite, B. M. (1977) *Dev. Biol.* **60**, 463–472
- Coleman, R. & Bell, R. M. (1976) *J. Biol. Chem.* **251**, 4537–4543
- Coleman, R. & Bell, R. M. (1977) *J. Biol. Chem.* **252**, 3050–3056
- Coleman, R. A. & Bell, R. M. (1980) *J. Biol. Chem.* **255**, 7681–7687

- Coleman, R. A. & Haynes, E. B. (1983a) *J. Biol. Chem.* **258**, 450–456
- Coleman, R. A. & Haynes, E. B. (1983b) *Biochim. Biophys. Acta* **751**, 230–240
- Coleman, R. A., Reed, B. C., Mackall, J. C., Student, A. K., Lane, M. D. & Bell, R. M. (1978) *J. Biol. Chem.* **253**, 7256–7261
- Diamant, Y. Z. & Shafir, E. (1972) *Biochim. Biophys. Acta* **279**, 424–430
- Galski, H., Fridovich, S. E., Weinstein, D., Degroot, N., Segal, S., Folman, R. & Hochberg, A. A. (1981) *Biochem. J.* **194**, 857–866
- Hahn, P. (1982) *Annu. Rev. Nutr.* **2**, 91–111
- Hull, D. & Elphick, M. (1979) in *Placental Transfer* (Chamberlain, G. V. P. & Wilkinson, A. W., eds.), pp. 159–165. Pitman Medical, Tunbridge Wells
- Hummel, L., Schirrmeister, W., Zimmermann, T. & Wagner, H. (1974) *Acta Biol. Med. Germ.* **32**, 311–314
- Hummel, L., Schirrmeister, W. & Zimmermann, T. (1975) *Acta Biol. Med. Germ.* **34**, 603–605
- Hummel, L., Schwartze, A., Schirrmeister, W. & Wagner, H. (1976) *Acta Biol. Med. Germ.* **35**, 1635–1641
- Karp, W., Sprecher, H. & Robertson, A. (1973) *Biol. Neonate* **22**, 398–406
- Kottel, R. H. & Fishman, W. H. (1981) *Biochem. J.* **200**, 679–684
- Lamb, R. G. & Fallon, H. J. (1974) *Biochim. Biophys. Acta* **348**, 166–178
- Lands, W. E. M. & Hart, P. (1965) *J. Biol. Chem.* **240**, 1905–1911
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mavis, R. D., Finkelstein, J. N. & Hall, B. P. (1978) *J. Lipid Res.* **19**, 467–477
- McComb, R. B. & Bowers, G. N. (1972) *Clin. Chem.* **18**, 97–104
- Merrill, A. H., Gidwitz, S. & Bell, R. M. (1982) *J. Lipid Res.* **23**, 1368–1373
- Mooney, R. A. & Lane, M. D. (1981) *J. Biol. Chem.* **256**, 11724–11733
- Moriyama, I. S., Ishibashi, S., Hiraoka, K., Ueda, S., Noda, T. & Yamaguchi, R. (1976) *Acta Obstet. Gynaecol. Jap.* **23**, 225–230
- Polokoff, M. A. & Bell, R. M. (1977) *J. Biol. Chem.* **252**, 1167–1171
- Schlossman, D. M. & Bell, R. M. (1976) *J. Biol. Chem.* **251**, 5738–5744
- Schlossman, D. M. & Bell, R. M. (1977) *Arch. Biochem. Biophys.* **182**, 732–742
- Schlossman, D. M. & Bell, R. M. (1978) *J. Bacteriol.* **133**, 1368–1376
- Sturton, R. G. & Brindley, D. N. (1978) *Biochem. J.* **171**, 263–266
- Szabo, A. J., deLellis, R. & Grimaldi, R. D. (1973a) *Am. J. Obstet. Gynecol.* **115**, 257–262
- Szabo, A. J., Grimaldi, R. D. & deLellis, R. (1973b) *Am. J. Obstet. Gynecol.* **115**, 263–266
- Winkel, C. A., Gilmore, J., MacDonald, P. C. & Simpson, E. R. (1980) *Endocrinology* **107**, 1892–1898