Perinatal hepatocyte/hepatoma hybrids: construction of clones that express the developmentally regulated monoacylglycerol acyltransferase activity

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Abstract Microsomal monoacylglycerol acyltransferase is a developmentally expressed enzyme that catalyzes the synthesis of sn-1,2-diacylglycerol from sn-2-monoacylglycerol and palmitoyl-CoA. The activity is present in liver from fetal and suckling rats but is absent in the adult. In order to obtain a stable permanent cell line that expresses this activity, Fao rat hepatoma cells and hepatocytes from 8-day-old baby rats were hybridized and clones were selected. Two hybrids (HA1 and HA7) expressed monoacylglycerol acyltransferase activity. Like fetal hepatocytes, but unlike hepatocytes from postnatal rats, the HA cells had high rates of [^{14}C]acetate incorporation into glycerolipids, cholesterol, and cholesteryl esters, and they secreted triacylglycerol into the media. Monoacylglycerol acyltransferase specific activity increased 2.5-fold as the cells divided in culture, suggesting growth-dependent regulation. The specific activities of glycerol-P acyltransferase, the committed step of the microsomal pathway of glycerolipid synthesis, and diacylglycerol acyltransferase, the activity unique to triacylglycerol biosynthesis, were comparable to the levels of the corresponding activities in fetal hepatocytes. Addition of insulin or dexamethasone to the media increased the incorporation of [^{14}C]oleate into triacylglycerol about 1.7-fold within 2 h, but had little effect on [^{14}C]oleate incorporation into phospholipid. ∎ These hormonally responsive rat-hepatoma/hepatocyte hybrids reflect the fetal stage of hepatocyte development in five major aspects of lipid metabolism: sterol, fatty acid, and triacylglycerol biosynthesis, glycerolipid secretion, and the presence of the developmentally expressed monoacylglycerol pathway. —**Coleman, R.A., and E. S-G. Bardes.** Perinatal hepatocyte/hepatoma hybrids: construction of clones that express the developmentally regulated monoacylglycerol acyltransferase activity. *J. Lipid Res.* 1990. **31:** 2257–2264.

Supplementary key words fetal hepatocytes • hybrid cells • monoacylglycerol acyltransferase • lipid metabolism • diacylglycerol

Hepatic monoacylglycerol acyltransferase is a developmentally expressed microsomal enzyme whose specific activity is 700-fold higher in postnatal rat liver than in the adult (1). In most eukaryotic cells, the major pathway of

glycerolipid biosynthesis begins with the acylation of glycerol-3-P to form phosphatidic acid which is at the branchpoint of the formation of the anionic phospholipids and sn-1,2-diacylglycerol (2). Diacylglycerol is the immediate precursor for the quantitatively most prominent glycerolipids: triacylglycerol, phosphatidylcholine, and phosphatidylethanolamine. Diacylglycerol can also be formed via minor catabolic pathways that include the regulated hydrolysis of phosphatidylinositol and phosphatidylcholine by phospholipase C. When diacylglycerol is formed via these latter catabolic routes in the plasma membrane, however, its role is thought to be limited to that of a second messenger and activator of protein kinase C.

Monoacylglycerol acyltransferase provides an alternate, and perhaps major, pathway of diacylglycerol synthesis during the perinatal period. The activity, which catalyzes the acylation of sn-2-monoacylglycerol, has a specific activity in postnatal rat liver about 300-fold higher than that of glycerol-3-P acyltransferase, the committed step of the glycerol-P pathway (1, 3). The activity is present in hepatocytes from perinatal rats, fetal guinea pigs, and embryo chicks, suggesting that it plays an important role in the developing animal (1, 4, 5). It remains unclear as to why this alternate route of diacylglycerol synthesis is required during perinatal life, and how the unusual developmental pattern is regulated. Long-term study of the regulation of this and other pathways of neonatal lipid metabolism have been precluded, not only

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because hepatocytes from fetal and neonatal rats are technically difficult to isolate, but also because, when grown in primary culture, hepatocytes rapidly lose differentiated functions (6).

Since a survey of several available permanent hepatoma and other lipogenic cell lines showed that none expressed the monoacylglycerol acyltransferase activity, we constructed a rat hepatocyte/hepatoma hybrid that retains many of the hormonally regulated lipogenic characteristics of fetal rat hepatocytes including the monoacylglycerol acyltransferase activity.

EXPERIMENTAL PROCEDURES

Materials

Fetal calf serum, Dulbecco's modified Eagle medium with 4.5 g glucose/l (DMEG) F-12 nutrient mixture, penicillin, streptomycin, and sterile tissue culture supplies were from Gibco. Collagenase was purchased from Worthington Biochemical Corp. Timed-pregnant Sprague-Dawley rats were from Zivic-Miller Laboratories, Inc. Zellenople, PA. Fao reuber hepatoma cells that were HGPRT-negative and ouabain-resistant were a generous gift from Dr. Emma E. Moore (Zymogen, Seattle, WA) (7). HepG2 cells and 3T3 L1 cells were obtained from the American Type Tissue Culture Collection. Dr. George Michalopoulos (Duke University) and Dr. James Neidel (Glaxo Inc., Durham, NC) kindly provided 4H2E, JB1, JM1, and JM2 hepatoma lines (8) and undifferentiated and phorbol-differentiated HL60 leukemia cells, respectively. Polyethylene glycol (approximately 1000 mol wt), hypoxanthine, aminopterin, thymidine, ouabain, dexamethasone, insulin, and bovine serum albumin (essentially fatty acid-free) were from Sigma. Silica gel G plates were from Analtech. [^{3}H]Palmitate, [^{3}H]glycerol, [^{14}C]acetate, and [γ-^{32}P]adenosine triphosphate were from New England Nuclear. [^{14}C]Oleic acid and [^{3}H]glycerol were from Amersham.

Methods

Hepatocyte isolation. To isolate hepatocytes from suckling rats, 8-day-old rats (17–20 g) were anesthetized with ether. PE 10 tubing was threaded from the right atrium into the inferior vena cava and secured above the liver. The inferior vena cava was clamped below the liver and the portal vein was severed to allow the perfusate to drain. Two to three ml of 1 mM EDTA in HEPES-buffered saline was flushed retrograde through the liver at a rate of 2 ml/min using a 10-ml syringe. Then 2.5 to 5.0 ml of 0.5 mg/ml collagenase in HEPES-buffered saline was infused manually at the same rate. The liver was gently removed, placed in fresh collagenase solution, and allowed to sit on ice until the process had been repeated for three livers (about 45 min). The livers were then teased apart gently

and filtered through 150 micron nylon mesh. The cells were rinsed three time in DMEG which contained 10% fetal bovine serum. About 4.3×10^7 cells were obtained per liver.

Construction of hybrids. Hybrid cells were obtained by a method similar to one previously described (9). Hepatocytes (6.5×10^6/dish) and Fao hepatoma cells (3×10^6/dish) were co-cultured in 14 100-mm culture dishes overnight in media that consisted of equal parts of DMEG and F-12, with 10% fetal bovine serum. All media routinely contained 100 U/ml penicillin and 100 μg/ml streptomycin. The cells were fused by adding 1 ml of 1 g/ml polyethylene glycol 1000 in serum-free DMEG at 37°C and then immediately aspirating the polyethylene glycol and rinsing the dishes twice with serum-free media at 37°C. The cells were then fed with DMEG, 16% fetal bovine serum, and 10 μg/ml insulin. After 48 h the cells were trypsinized and replated at a 1:4 dilution in selective media (1.0 mM ouabain, 0.1 mM hypoxanthine, 0.4 μM aminopterin, 16 μM thymidine, 16% fetal bovine serum, 10 μg/ml insulin, and DMEG). The media were replaced weekly for 1 month. Insulin was omitted after the first week. After 1 month, DMEG with 15% fetal bovine serum was used. Unfused control cells (Fao cells co-cultured with primary hepatocytes) that had been treated identically died during this time.

Ninety-nine clones were isolated and subcloned. When each clone was nearly confluent, the dishes were rinsed with and scraped into cold medium 1 (0.25 M sucrose, 10 mM Tris, pH 7.4, 1 mM EDTA), and homogenized with 10 up-and-down strokes in a motor-driven Teflon glass homogenizer at moderate speed. Total particulate preparations were obtained by centrifuging the homogenates at 100,000 g for 1 h and monoacylglycerol acyltransferase activity was measured in each preparation.

Cell culture and labeling. Cells were plated at about 4×10^6 cells per 60-mm culture dish in 3 ml of DMEG, 10 μg/ml insulin, and 10% fetal bovine serum and incubated at 37°C in 5% CO$_2$, 95% air. The medium was routinely changed every 2 or 3 days. Hybrid cells were passaged before reaching confluence to avoid the development of multilayered colonies. Unless otherwise indicated, labeling experiments were begun by replacing the medium with DMEG, 1 mM pyruvate, 10 μg/ml insulin, and 10 mg/ml bovine serum albumin for 24 h in order to deplete glycerolipid precursors and promote synthesis. The medium was then replaced with the same medium containing 100 μM [^{14}C]acetate (11 μCi/μmol) or 100 μM [^{14}C]oleate (0.25 μCi/μmol). After various times of incubation, the medium was removed and saved. The cells were then washed with 1 ml calcium-free Hank's balanced salts containing 10 mg/ml albumin, and the wash was added to the saved media. The cells were then scraped from the dish in cold methanol and the products

were extracted into $CHCl_3$ by the method of Bligh and Dyer (10) as described previously (11). The medium was similarly extracted. An aliquot of the $CHCl_3$ phase was counted and the remainder was concentrated under N_2. The products were identified by thin-layer chromatography on 10-cm silica gel G plates developed in heptane–isopropyl ether–acetic acid 60:40:4 (v/v).

Other methods. Protein was measured by the method of Lowry et al. (12) using bovine serum albumin as the standard. Fatty acid CoA ligase (13), diacylglycerol acyltransferase (3), monoacylglycerol acyltransferase (1), and total glycerol-3-P acyltransferase (14) activities were determined by radiochemical methods in assays that measured initial rates. [³H]Palmitoyl-CoA (15) and [³H]glycerol-3-P (14) were synthesized by previously reported methods.

RESULTS

In order to find a permanent cell line that expressed monoacylglycerol acyltransferase activity, we examined total particulate preparations from six hepatoma lines (HepG2, FAO reuber, 4H2E, JB1, JM1, and JM2), from 3T3-L1 fibroblasts before and after adipocyte differentiation was induced by dexamethasone, methylisobutylxanthine, and insulin (16), and from HL60 leukemia cells before and after differentiation was induced by phorbol esters. In these cells, the specific activities of monoacylglycerol acyltransferase were all less than 0.5 nmol/min per mg of protein, a level similar to that observed in adult hepatocytes. Therefore, we constructed hepatocyte/hepatoma hybrid cells in order to obtain cells with the characteristics of lipid biosynthesis that are present in perinatal hepatocytes.

Four of the initial hybrid subclones had monoacylglycerol acyltransferase specific activities that ranged between 0.9 and 7.0 nmol/min per mg of total particulate protein. The clone (HA) which had the highest monoacylglycerol acyltransferase activity was subcloned again and two of the resulting subclones, HA1 and HA7, were selected for further study.

At passage 16 HA1 and HA7 had average chromosome numbers of 65 (range 54–69) and 66 (range 61–92), respectively. The parent Fao cells have an average of 52 chromosomes (7), whereas rat hepatocytes have 42. Thus, the HA cells had fewer chromosomes than the sum of the two parent cells. When Fao cells were hybridized with hepatocytes from adult rats, the average chromosome number ranged from 66 to 117 (9).

Growth characteristics of the HA1 and HA7 hybrids differed from the parent Fao line. After growing in culture for 3 or 4 days, the HA hybrid cells piled up in clusters whereas the Fao parent line continued to grow in a spreading monolayer. When injected subcutaneously into nude mice at equal concentrations (10^6 cells/site), the HA hybrids formed tumors that expressed high monoacylglycerol acyltransferase specific activities (HA7: 22.2 and 11.5 nmol/min per mg of total particulate protein [two tumors], HA1: 10.6 to 17.9 nmol/min per mg [four tumors]). HA7 tumors, which weighed more than 0.5 g by 15 days after injection, were the fastest growing. HA1 tumors reached a similar size at 26 days, whereas the Fao cells formed equivalent sized tumors 36 days after injection.

The specific activities of monoacylglycerol acyltransferase were 3.5 and 14.3 nmol/min per mg of total particulate protein in HA7 and HA1, respectively, which was comparable to the activity observed in microsomes from fetal rats (**Table 1**). HA cells expressed the hepatic isoenzyme which is highly selective for *sn*-2-monoacylglycerols; in contrast to monoacylglycerol acyltransferase from intestinal mucosa, the hepatic isozyme acylates 1- and 2-monoalkylglycerols poorly (17). Both HA lines retained monoacylglycerol acyltransferase activity for more than 30 successive passages. Monoacylglycerol acyltransferase activity rose in HA7 cells as they grew in culture, reaching a maximum as the amount of protein per dish plateaued (**Fig. 1**). Although the amount of cellular protein did not increase after the first 5 days in culture, the specific activity continued to rise during the next five days from 4.65 to 6.9 nmol/min per mg of total particulate protein. In subsequent studies, assays were performed on the third to the fifth day after passage when cells were about three-fourths confluent and beginning to pile up after having been plated at a usual density of about 4×10^6 cells/60 mm dish.

The hybrid cells were also examined for several critical glycerolipid synthetic enzyme activities and these were compared with the parent Fao and hepatocyte cells (Table 1). In HA cells, the specific activity of glycerol-3-P

TABLE 1. Glycerolipid synthetic activities in HA and Fao cells compared with activities in rat liver and hepatocytes

	MGAT	GPAT	DGAT	Ligase
	nmol/min/mg protein			
HA 7[a]	3.5	1.2	3.8	3.3
HA 1[a]	14.3	3.0	6.4	5.0
Fao[a]	<0.5	2.3	1.4	4.8
8-Day hepatocytes[a,b]	88.4	2.0	5.8	31.4
Fetal microsomes[c]		0.5	3–7	
8-Day microsomes	221 ± 56[d]	1.5[c]	2.4 ± 0.6[d]	25[c]
Fetal liver[a,e]	9.3 ± 4.4			

MGAT, monoacylglycerol acyltransferase; GPAT, glycerol 3-P acyltransferase; DGAT, diacylglycerol acyltransferase; Ligase, fatty acid CoA ligase.
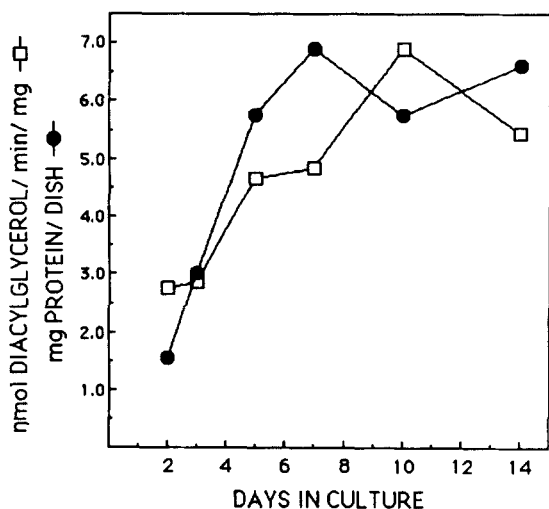[a]Total particulate protein.
[b]Data from Reference 17.
[c]Data from Reference 3.
[d]n = 5.
[e]Data from Reference 1.

Fig. 1. Changes in monoacylglycerol acyltransferase specific activity during culture of HA7 cells. Approximately 10^6 cells were plated in each 100-mm dish in DMEG, 10% FBS, and 10 µg/ml insulin. The medium was changed every 2 days. On alternate days, cells from each of two plates were rinsed with cold phosphate-buffered saline and scraped and homogenized on ice-cold Medium 1. Protein concentration and enzyme activity were measured in the cell homogenates and data are plotted as the average of two determinations.

acyltransferase, the committed step of glycerolipid biosynthesis, was similar to both parental cell lines, whereas diacylglycerol acyltransferase, the activity unique to triacylglycerol synthesis, was 2.7- to 4.6-fold higher in the hybrid cells than in the Fao parent line. The specific activity of fatty acid CoA ligase, which activates long-chain fatty acids, was similar in the three permanent cell lines but more than 6-fold higher in the parent hepatocytes.

Lipid metabolism in fetal and postnatal rat hepatocytes can be functionally distinguished. Fatty acid synthase and HMG-CoA reductase activities are high in fetal liver, as is the rate of acetate incorporation into fatty acids and cholesterol (18, 21). The precipitous decrease of these activities after birth coincides with the advent of suckling and the high intake of dietary cholesterol and triacylglycerol from rat milk. These dietary changes are thought to alter hepatic gene expression, either directly or through an indirect mechanism that is controlled by changes in plasma hormone concentration (22).

In order to determine whether the lipid metabolism of the HA cells was more comparable to that of fetal or of postnatal rat hepatocytes, we measured the incorporation of labeled substrates into lipid. HA1, HA7, and the parent Fao cells were incubated with [^{14}C]acetate and, after various times of incubation, the products were extracted into $CHCl_3$ and identified. During a 6-h labeling period, acetate was incorporated into cellular phospholipid, triacylglycerol, sterol, and cholesteryl esters (**Fig. 2**). Less than 1% of the label in cells was recovered as free fatty

acid. At 4 h, HA7 and HA1 cells incorporated 1.7 and 3.0 times, respectively, more acetate into triacylglycerol than did the Fao cells. Compared to the Fao cells, both HA lines decreased the amount of acetate incorporated into phospholipid. Even though they had been subcloned from the same original HA clone, HA1 and HA7 differed in the amount of acetate incorporated into triacylglycerol versus phospholipid. Their synthetic rates for sterol and cholesteryl ester, however, remained similar.

The high incorporation of acetate into triacylglycerol and phospholipid by both of the HA cell lines was similar to that seen in fetal hepatocytes (**Fig. 3A**) and liver slices from fetal rats (18); incorporation into triacylglycerol was more than 6-fold higher than observed in hepatocytes from 6-day-old rats (Fig. 3A) and more than 15-fold higher than reported in hepatocytes from 12-day-old rats (23). Little acetate incorporation occurs postnatally
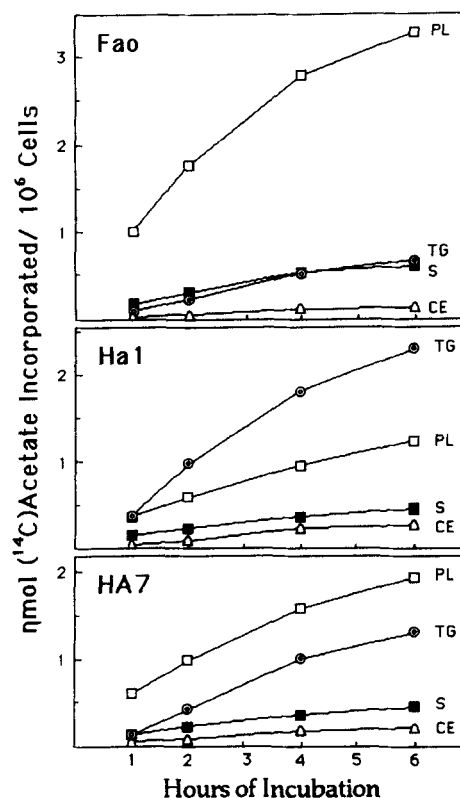
Fig. 2. Incorporation of [^{14}C]acetate into glycerolipids and sterols by HA1, HA7, and Fao cells. HA1 (2.5×10^6), HA7 (2.6×10^6), and Fao cells (2.5×10^6) were seeded in DMEG containing 10% FBS and 10 µg/ml insulin. After 24 h the medium was changed to DMEG containing 1 mM pyruvate, 10 µg/ml insulin, and 10 mg/ml bovine serum albumin. After 24 h the medium was replaced with the same medium that contained 100 µCi [^{14}C]acetate (1 µCi/µmol). At the time points indicated, the cells were washed with calcium-free Hanks buffered salt solution. Products were extracted and identified as described under Experimental Procedures. The number of cells/dish was counted in comparably treated dishes at the end of the incubations. Each point represents the mean from three dishes. PL, phospholipid; TG, triacylglycerol; S, sterol; CE, cholesteryl ester.
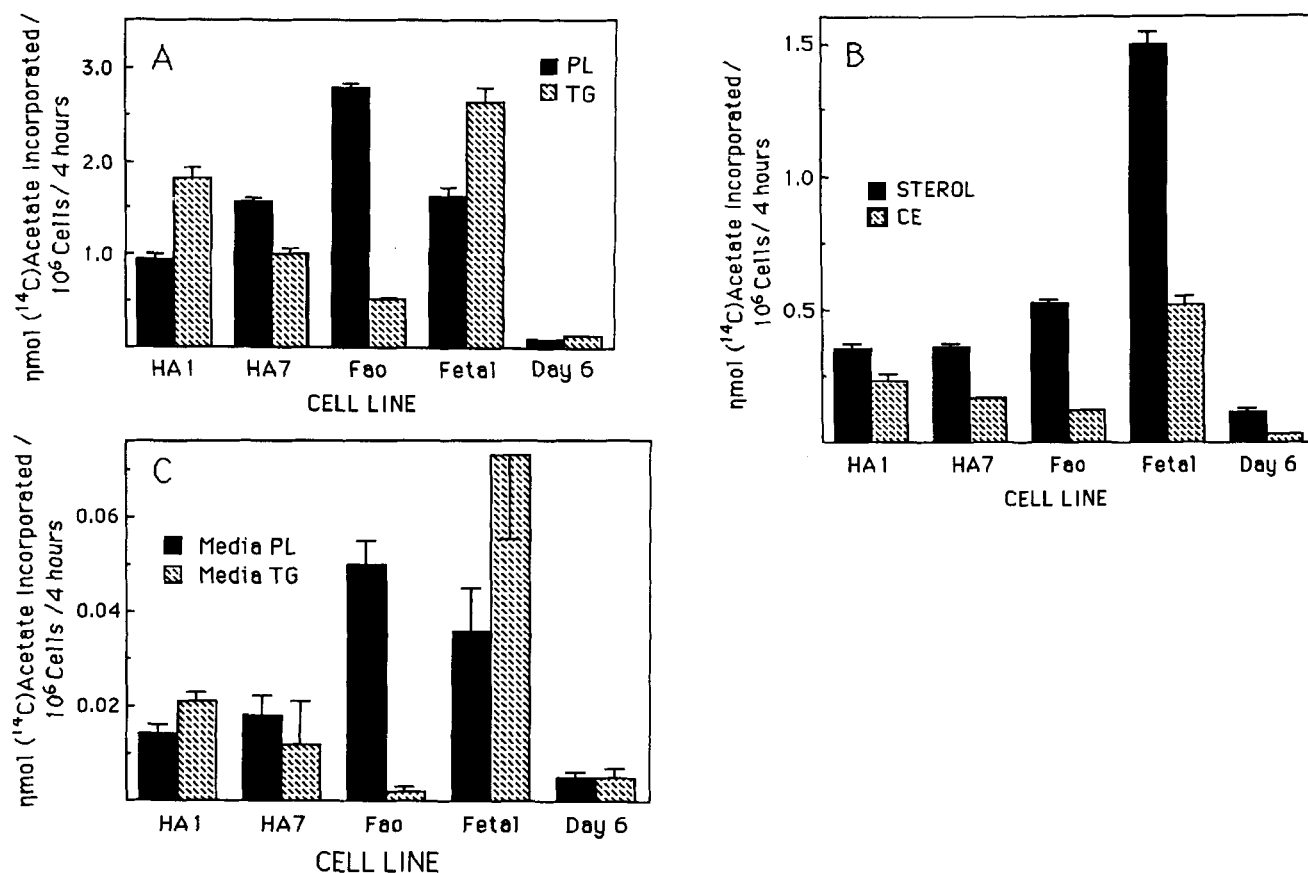
Fig. 3. Incorporation of [^{14}C]oleate into (A) cellular triacylglycerol and phospholipid, (B) cellular sterol and cholesteryl esters, and (C) secreted triacylglycerol and phospholipid at 4 h in HA1, HA7, Fao, fetal hepatocytes, and hepatocytes from 6-day-old rats. Incubation conditions for HA1, HA7, and Fao cells are described in the legend to Fig. 2. Comparative data for fetal and day-6 hepatocytes are taken from ref. 17. Data are plotted as the mean ± SD of three dishes; where absent, the SD was too small to plot.

because fatty acid synthase activity is low during the suckling period.

Like fatty acid synthase, HMG-CoA reductase activity also decreases postnatally (21, 24). This postnatal decrease in HMG-CoA reductase activity is reflected in the amount of acetate incorporated into sterols by fetal and day-6 hepatocytes (Fig. 3B). Like the parent Fao line, the incorporation of [^{14}C]acetate into sterols and cholesteryl esters was relatively high compared to day-6 hepatocytes. Thus, like the rate of acetate incorporation into triacylglycerol, the rate of sterol synthesis by HA cells was more like their Fao parent than their hepatocyte parent (Fig. 3A, B).

Although Fao cells secrete VLDL (25, 26), their ability to secrete triacylglycerol into the media was limited compared with fetal hepatocytes (Fig. 3C). HA cell secretion of triacylglycerol, on the other hand, was higher than that of the Fao cells and intermediate between that of fetal and postnatal hepatocytes (Fig. 3C). Sterol secretion for each of the three cell lines (about 0.01 nmol/10^6 cells per 4 h), was similar to that reported for fetal hepatocytes (23). Little cholesteryl ester (< 1.0 pmol/10^6 cells) was secreted by either the hybrid cells or the Fao parent.

Whereas acetate incorporation into glycerolipids reflects fatty acid synthesis, oleate incorporation depends on the microsomal enzymes of glycerolipid synthesis. The HA hybrids incorporated [^{14}C]oleate into phospholipid and triacylglycerol at rates higher than observed for fetal hepatocytes (**Fig. 4**). The low fetal rate may reflect the relatively low specific activity of the first step in the pathway, the glycerol-3-P acyltransferase (Table 1).

In order to determine whether the hybrid cells could respond to two hormones that have been shown to alter the expression of several hepatic enzymes during the perinatal period, we examined [^{14}C]oleate incorporation by HA1 cells after they were incubated with insulin, dexamethasone, or both hormones together (**Fig. 5**). Insulin increased the incorporation of [^{14}C]oleate into triacylglycerol about 1.8-fold at 2 and 4 h but had little effect on cellular phospholipid. Dexamethasone had a similar effect, increasing incorporation into triacylglycerol 1.7-fold at 2 h. When both hormones were added together, oleate incorporation into triacylglycerol increased about 2.0-fold. During the 4-h incubation, the hormone treatment did not alter the small amount of glycerolipid released into the media.
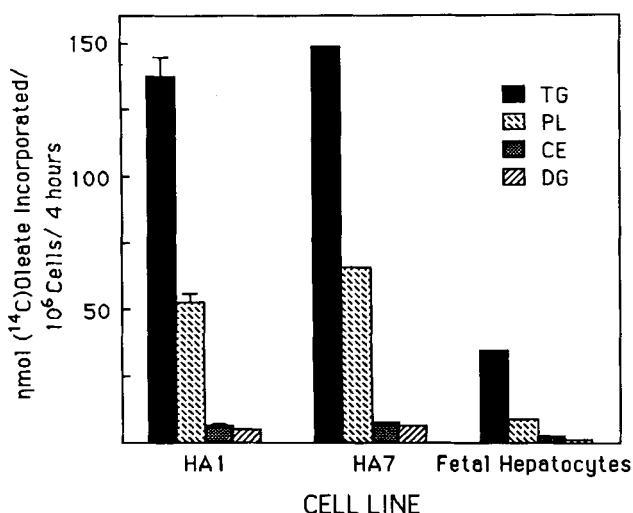
Fig. 4. Incorporation of [^{14}C]oleate into triacylglycerol, phospholipid, cholesteryl ester, and diacylglycerol. HA1 and HA7 cells were grown in DMEG containing 10 µg/ml insulin and 10% FBS. The medium was then replaced with DMEG containing 1.0 mM [^{14}C]oleate (0.25 µCi/µmol), 10 µg/ml insulin, and 10 mg/ml bovine serum albumin. After 4 h, the cell lipids were extracted and identified as described under Experimental Procedures. Data for HA1 are plotted as the mean ± SD for three dishes. For HA7 and fetal hepatocytes, data are plotted as the average of two dishes. HA7 and HA1 cells secreted 4.5 and 15.5 nmol [^{14}C]triacylglycerol/10^6 cells per 4 h, respectively.

DISCUSSION

During mammalian development hepatocytes are progressively programmed to express at least three distinguishable constellations of biochemical activities. These three independent patterns correspond to the late fetal, suckling, and post-weaning stages of development, and each stage is characterized by a unique cluster of enzyme activities and the synthesis of specific proteins (22).

Study of hepatocyte metabolism at each of the early developmental stages has proceeded slowly because of the paucity of permanent hepatocyte cell lines that retain differentiated activities that can be specifically modulated. The use of isolated hepatocytes in primary culture is fraught with problems of reproducibility from one preparation to another, loss of cell membrane receptors during the collagenase perfusion, and altered expression of differentiated enzymes and functions after several days in culture. These characteristics of primary cell cultures have precluded studies of the long-term regulation and molecular reprogramming that occurs during fetal and postnatal development. In addition, the study of metabolic processes that are present only during the fetal or postnatal periods, requires that cells be obtained from very small animals; perfusing the tiny neonatal liver is technically difficult, and multiple livers must be perfused in order to isolate enough hepatocytes.

In the rat, monoacylglycerol acyltransferase activity is present in late fetal liver, reaches its peak activity postnatally, and declines to virtually unmeasurable levels shortly after weaning (1). Because we wished to study the function and regulation of this enzymatic marker of the fetal and suckling stages of hepatocyte programming, we constructed permanent hybrid lines that retain the monoacylglycerol pathway of diacylglycerol synthesis.

Several laboratories have constructed immortalized cell lines in order to obtain cells that express specific differentiated functions. Using hybridization methods similar to those outlined above, investigators have constructed hepatocyte lines that synthesize and secrete bile acids (9), Leydig cells that secrete testosterone (27), and other cell lines that secrete human factor VIII-related protein (28), glucagon (29), or progesterone (30). For our studies, the Fao reuber rat hepatoma line was used as the fusion partner because Fao cells grow clonally and are relatively well-differentiated. They secrete albumin, have hormone-inducible tyrosine and alanine aminotransferases, and contain the liver-specific isoenzymes of aldolase and alcohol dehydrogenase (31). During hybrid construction, the
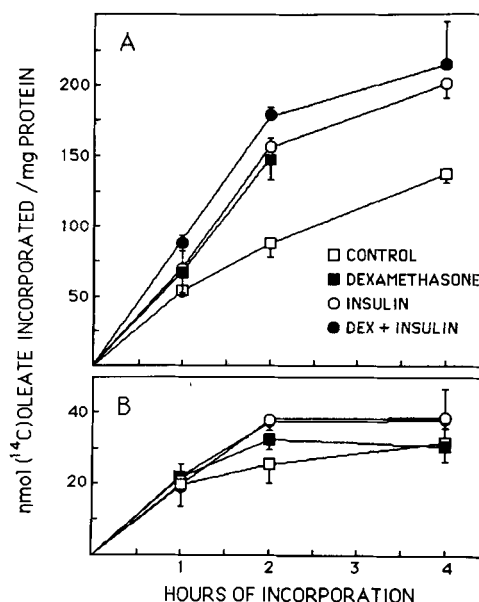
Fig. 5. Effect of insulin and dexamethasone on the incorporation of [^{14}C]oleate into A) triacylglycerol and B) phospholipid. HA1 cells were plated at approximately 15,000 cells/well in 24-well tissue culture dishes. After 3 days in culture in DMEG containing 10% FBS, fresh medium containing 10 µg/ml insulin, 0.1 µg/ml dexamethasone, or both insulin and dexamethasone was added to the dishes. The following day fresh medium containing 5% FBS, 10 mg/ml bovine serum albumin, hormone, and 1.0 mM [^{14}C]oleate (1.7 µCi/µmol) was added. At the intervals indicated, the monolayer was washed 3 times with phosphate-buffered saline. The products were extracted and identified as described under Experimental Procedures. The data are plotted as the mean ± SD for three determinations of a representative experiment that was performed three times.

use of a permanent cell line from the same species is said to reduce the extinction of tissue-specific functions of the primary cell line (32, 33). Even so, of the 99 hybrid clones obtained in this study, only 4 retained the monoacylglycerol acyltransferase activity expressed by the hepatocyte parent.

Although the hybrid HA cell lines were derived from postnatal hepatocytes, both of the HA lines expressed monoacylglycerol acyltransferase with a specific activity comparable to the level of activity observed in fetal hepatocytes. HA cells also expressed other major lipogenic properties which characterize fetal hepatocytes: a high rate of acetate incorporation into sterols, cholesteryl esters, and glycerolipids, and a relatively low rate of triacylglycerol secretion. Glycerolipid synthesis in the HA hybrid cell lines appears to respond to both insulin and dexamethasone, two hormones that have been shown to regulate specific events of biochemical maturation in fetal hepatocytes. Thus, in the HA hybrids, monoacylglycerol acyltransferase activity, lipogenesis, and responsivity to hormones reflect the late fetal stage of hepatocyte programming. These hybrid cells should be useful in furthering the study of the developmentally regulated hepatic monoacylglycerol acyltransferase as well as the program of lipogenesis which is characteristic of fetal hepatocytes. ▪

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