Hepatic monoacylglycerol acyltransferase: ontogeny and characterization of an activity associated with the chick embryo

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Abstract Hepatic monoacylglycerol acyltransferase is expressed during the perinatal period in rats and guinea pigs and appears to be related temporally to the availability of fatty acids and to the development of hepatic steatosis. In order to determine when monoacylglycerol acyltransferase activity is expressed in an avian species, its ontogeny was investigated in chick liver total particulate preparations. In livers from 11- to 21-day-old chick embryos, monoacylglycerol acyltransferase specific activity was 34.5 ± 8.1 nmol/min per mg of total particulate protein. The specific activity decreased 93% to 2.6 ± 1.3 nmol/min per mg by the 6th day after hatching. The specific activities of fatty acid CoA ligase, diacylglycerol acyltransferase, and microsomal and mitochondrial glycerol-P acyltransferases changed comparatively little during this time period. In the embryos, the monoacylglycerol acyltransferase activity per liver rose 28-fold between the 11th and 21st day, corresponding exactly to the increase in liver total particulate protein during this time. Monoacylglycerol acyltransferase activity in other tissues was 25- to 115-fold lower than observed in liver. Optimal activity was measured using 25 μM palmitoyl-CoA and 50 μM *sn*-2-monooleoylglycerol. The activity with the 1- and 2-monooleoylglycerol ethers and 1-monooleoylglycerol was very low. In contrast to microsomes from rat liver, about 70% of the product with the 1- and 2-monooleoylglycerol ethers was triradylglycerol, suggesting that the diacylglycerol acyltransferase from chick liver can acylate acyl, alkylglycerols. The activity with *sn*-2-monooleoylglycerol amide was 12.5% of that observed with the corresponding 2-monooleoylglycerol suggesting that the ester bond is important; the 1-monooleoylglycerol amide was not a substrate. The association of chick embryo liver with monoacylglycerol acyltransferase activity suggests that the enzyme plays a role in diacylglycerol synthesis during this time period. Monoacylglycerol acyltransferase activity was unresponsive to fasting, suggesting that the decrease in specific activity after hatching is independent of both diet and the hormones implicated in the regulation of fatty acid synthase. —**Sansbury K., D. S. Millington and R. A. Coleman.** Hepatic monoacylglycerol acyltransferase: ontogeny and characterization of an activity associated with the chick embryo. *J. Lipid Res.* 1989. **30:** 1251–1258.

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In most tissues, the major route for the synthesis of diacylglycerol and the quantitatively most significant glycerolipids, triacylglycerol, phosphatidylcholine, and phosphatidylethanolamine, occurs via the sequential acylation of glycerol-3-P. However, in intestinal mucosa throughout life (1) and in liver from rats and guinea pigs during the neonatal period (2,3) the monoacylglycerol pathway provides the major route. In rats, the intestinal and liver monoacylglycerol acyltransferase activities appear to be separate isoenzymes with differing ontogenies and substrate specificities (4). Hepatic monoacylglycerol acyltransferase is highly specific for *sn*-2-monoacylglycerol, whereas the intestinal activity readily acylates *sn*-1-monoacylglycerol and the ether analogs of 1- and 2-monoacylglycerol (4).

It is as yet unclear what role the monoacylglycerol pathway plays in neonatal liver. In rats the specific activity of monoacylglycerol acyltransferase peaks during the second postnatal week and then falls to virtually unmeasurable levels in the adult (2). In the guinea pig, on the other hand, monoacylglycerol acyltransferase activity peaks during the last third of gestation and decreases at birth (3). Both of these ontogenic patterns correspond to developmental periods when the influx of lipid is high: after birth, the rat receives 70% of its calories from milk triacylglycerol, and during last third of gestation, the fetal guinea pig receives a large amount of fatty acid which has been transported by the placenta (5–7). We wondered whether the hepatic monoacylglycerol acyltransferase activity in fowl would correspond to the time period when egg yolk lipids are available, or whether the activity is nutritionally induced after hatching like the enzymes that are required

Abbreviations: NEM, n-ethylmaleimide; EDTA, (ethylene dinitrilo)-tetraacetic acid; DTT, dithiothreitol.

for the synthesis of fatty acids (8,9). In order to answer this question, we examined the ontogeny of monoacylglycerol acyltransferase from chick liver and characterized the activity from this source.

EXPERIMENTAL PROCEDURES

Materials

Bovine serum albumin (essentially fatty acid-free), *N*-ethylmaleimide (NEM) and L-α-glycerophosphate were from Sigma. Phosphatidylcholine, phosphatidylserine, *rac*-1(3)- and *sn*-2-monooleoylglycerols, and *rac*-1(3)- and *sn*-2-monooleoylglycerol ethers were from Serdary. Palmitoyl-CoA was from P. L. Biochemicals. Aquasol-2, [^{3}H]palmitic acid, and [^{3}H]glycerol were from New England Nuclear. [^{3}H]Palmitoyl-CoA (10), [^{3}H]glycerol-3-phosphate (11), and *rac*-1(3)- and *sn*-2-monooleoylglycerol amides (12) were synthesized by previously reported methods.

Methods

Fertilized Arbor Acre chicken eggs were obtained from the North Carolina State University Poultry Science Department and were timed from the first day of incubation. After hatching (on day 22), the chicks were allowed free access to water and Pullet Grower feed. On selected days, embryos and chicks were decapitated and the livers were rapidly removed. Livers were minced and homogenized with ten up-and-down strokes in a motor-driven Teflon–glass homogenizer at moderate speed in ice-cold medium 1 (0.25 M sucrose, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4). Total particulate preparations were obtained by centrifuging the homogenate at 100,000 *g* for 1 h. To obtain a microsomal preparation, the homogenate was centrifuged at 1000 *g* for 10 min and the pellet was discarded. The supernatant was centrifuged at 25,000 *g* for 10 min. The resulting pellet was discarded and the supernatant was centrifuged at 100,000 *g* for 1 h. The resulting microsomal pellet was resuspended in medium 1 and stored at −70°C in small samples. Protein was determined using bovine serum albumin as the standard (13).

Enzyme assays

Monoacylglycerol acyltransferase activity was measured using 25 μM [^{3}H]palmitoyl-CoA and 50 μM *sn*-2-monooleoylglycerol dispersed in acetone (3). The assay mixture of 200 μl routinely contained 1 mg/ml bovine serum albumin, 100 mM Tris-HCl, pH 7.0, 5 μl of acetone, and 15 μg of a 1:1 (w/w) mixture of phosphatidylcholine and phosphatidylserine sonicated in 10 mM Tris-HCl, pH 7.0. The assay was begun with the addition of 1–2.5 μg of microsomal or total particulate protein. After a 5-min incubation at 23°C, the products were extracted into heptane and washed one time (14). An aliquot of the heptane phase was counted and the remainder was dried in a Speed-Vac concentrator and chromatographed on 10-cm silica gel G plates (Analtech) in heptane–isopropyl ether–acetic acid 30:20:2 (v/v) with carrier lipids. Lipids were visualized by exposing the plates to iodine vapor. The areas corresponding to diacylglycerol and triaclyglycerol were scraped and counted. Monoacylglycerol acyltransferase specific activities were calculated by subtracting one-half the cpm which appeared as triacylglycerol. Diacylglycerol and triacylglycerol comprised more than 98% of the labeled product.

Fatty acid CoA ligase was measured using 50 μm [^{3}H]palmitate and 5 mM ATP (15). Diacylglycerol acyltransferase was measured using 30 μM [^{3}H]palmitoyl-CoA and 200 μM *sn*-1-2-dioleoylglycerol dispersed in acetone (14). In order to distinguish between the microsomal (NEM-sensitive) and mitochondrial (NEM-resistant) glycerol-P acyltransferase activities, membrane fractions were incubated for 15 min at 4°C in the presence or absence of N-ethylmaleimide and aliquots were removed for assay (11). Glycerol-P acyltransferase activity was assayed using 112.5 μM palmitoyl-CoA and 300 μM [^{3}H]glycerol-3-P (11). Products of the glycerol-P acyltransferase activity were analyzed by thin-layer chromatography on 20-cm silica gel G plates developed in chloroform–pyridine–88% formic acid 50:30:7 (v/v) and were 66% phosphatidic acid and 30% lysophosphatidic acid. Enzyme assays were performed at 23°C and were proportional to the time and the amount of protein employed. The substrate concentrations used gave maximal activities.

Amide products

Microsomes from 15-day embryos were incubated for 10 min with 75 μM *sn*-2-monooleoylglycerol amide and 112.5 μM palmitoyl-CoA. The products were extracted into hepane, dried under N_2, and separated by thin-layer chromatography as described in Methods. The R_f values were 0.03 and 0.25, for di- and tri-radylglycerols, respectively. Each area was scraped into a screw-cap tube and extracted with 3 ml chloroform–methanol 1:2 (v/v), 0.6 ml 1% $HClO_4$, and 0.2 ml H_2O for 1 h. After breaking phases with 1 ml 1% $HClO_4$ and 1 ml $CHCl_3$, the $CHCl_3$ phase was dried under N_2. The diradylglycerol was derivitized with bis(trimethylsilyl)-trifluoracetamide and the triradylglycerol was dissolved in $CHCl_3$.

Mass spectrometric analyses were performed on a VG 70-S/11-250 high resolution mass spectrometer/data system (VG Analytical, Manchester, UK) using the direct-insertion probe for sample introduction. Low-resolution mass spectra were obtained by scanning from *m/z* 1000 to 50 in 5s (electron energy, 70 eV; source temperature, 190°C). Accurate mass measurements were performed by peak-matching at a resolution of 10000 (10% valley definition) against ions of known mass in perfluorokerosene.

RESULTS

Ontogeny of hepatic monoacylglycerol acyltransferase

In livers from 10- to 21-day-old chick embryos, monoacylglycerol acyltransferase specific activity was 34.5 ± 8.1 nmol/min per mg of total particulate protein. (**Fig. 1B**). After hatching, the specific activity fell to 2.6 ± 1.3 nmol/min per mg by the sixth day after hatching. In the embryos, total monoacylglycerol acyltransferase activity per liver rose 28-fold between days 10 and 21 (Fig. 1A), corresponding in large part to the increase in total particulate protein during this time (**Fig. 2**). Because total particulate protein increased a further 9.1-fold between hatching and day-6, total monoacylglycerol acyltransferase activity per liver did not change after hatching despite the decrease in specific activity (Fig. 1B). Mixing experiments using total particulate preparations from a day-15 embryo and a 6-day-old chick showed additive monoacylglycerol acyltransferase activity, indicating that no activator or inhibitor was present.

Characterization of hepatic monoacylglycerol acyltransferase

Since hepatic monoacylglycerol acyltransferase has been previously characterized only in neonatal rats (2), we initiated studies on the activity from chick embryo liver microsomes. The activity was proportional to the amount of microsomal protein to 2.5 μg at 5 min and with time to 15 min when 1.5 mg of protein was used (data not shown). More than 98% of the labeled products were identified as diacylglycerol and triacylglycerol by thin-layer chromatography. The percent of triacylglycerol synthesized rose to a maximum of 75% after a 15-min incubation. (**Fig. 3**).

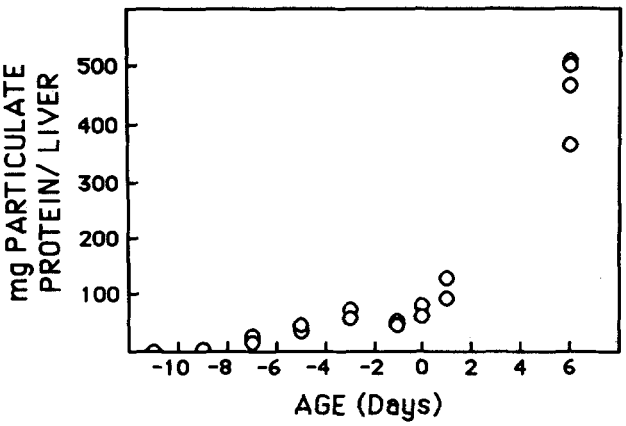

Fig. 2. Time course showing changes in liver total particulate protein in chick embryos and chicks. Each point represents a determination from one to four combined livers. Day -1 corresponds to a 21-day embryo.

Dependencies of monoacylglycerol acyltransferase activity

Since the pH optimum plateaued between 7.0 and 8.0, assays were routinely performed at pH 7.0 in order to minimize acyl-migration (16–18). The activity was completely dependent on the addition of microsomes. Optimal activity was measured using 25 μM palmitoyl CoA

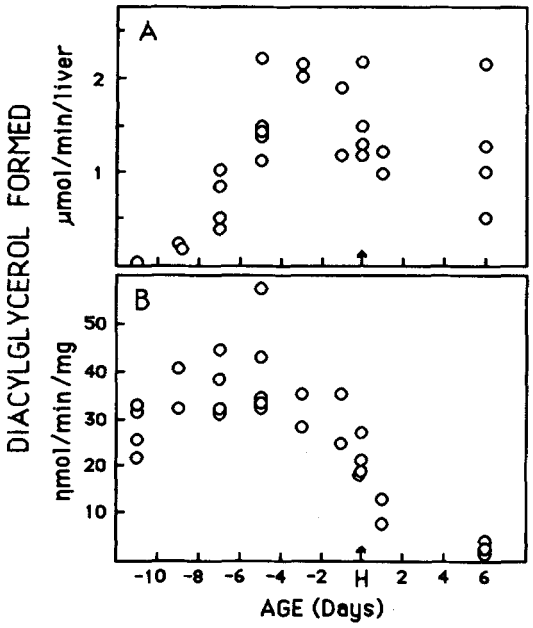

Fig. 1. Time course showing changes in (A) total activity and (B) specific activity of monoacylglycerol acyltransferase in liver total particulate fractions from chick embryos and chicks. Each point represents a determination from one to four combined livers. H, hatch. Day -1 corresponds to a 21-day embryo.

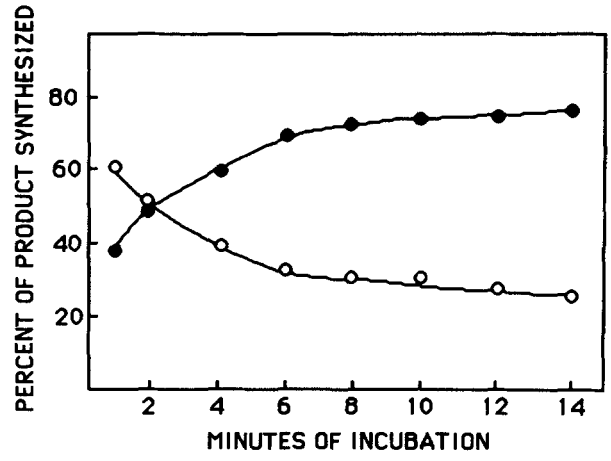

Fig. 3. Products of monoacylglycerol acyltransferase with time. Hepatic microsomes from a 3-day-old chick were incubated as described under Methods. At the indicated times, the labeled products were extracted and identified by thin-layer chromatography. At 4 min the specific activity was 6.5 nmol/min per mg.

(**Fig. 4**). Although the calculated apparent K_m for palmitoyl-CoA was 44 μM, high concentrations of the substrate were inhibitory. In contrast to their large stimulatory effects on monoacylglycerol acyltransferase from rat liver (2), 1 mg/ml bovine serum albumin and the phosphatidylcholine/phosphatidylserine mixture stimulated the chick liver activity less than 10%. Monoacylglycerol added in 5 or 10 μl acetone gave similar activities, but the addition of 20 μl acetone (10% of the final assay volume) decreased activity by 30%. At 2.5 mM, $HgCl_2$ inhibited activity 90%. KI at 350 mM or $CaCl_2$ at 10 mM inhibited the activity 30 and 35%, respectively. $MnCl_2$ at 2 mM and $MgCl_2$ at 16 mM inhibited the activity 20 and 15%, respectively. No change in activity occurred when microsomes were assayed in the presence of 2 mM glycerol-3-P, 1.5 mM CDP-choline, 1 mM citrate, or 1 mM carnitine. The activity was unaffected by a variety of salts and chelators including 5 mM EDTA, 8 mM NaF, 7.5 mM DTT, 3.5 mM NEM, 400 mM NaCl, 200 mM KCl, or 2 mM EDTA.

Monoacylglycerol dependencies

Monoacylglycerol acyltransferase activity was greatest with *sn*-2-mono oleoylglycerol (**Fig. 5**). The activity acylated 1- and 2-monooleoylglycerol ethers, and 1-monooleoylglycerol very poorly. It is unlikely that much, if not all, of the low activity observed with 1(3)-monooleoylglycerol was due to small amounts of 2-monoacylglycerol that had arisen by acyl-migration as was shown for the activity from rat liver (20). In order to determine whether the O-bond was essential, the 1(3)- and 2-monooleoylglycerol amides were synthesized (12) and tested. Only the *sn*-2-monooleoylglycerol amide was a substrate. With palmitoyl-CoA the glycerol amide products, identified by fast atom bombardment-mass spectrometry, were 1-palmitoyl, 2-oleoyl amide glycerol, and 1,3-dipalmitoyl, 2-oleoyl-amide glycerol (**Fig. 6 and Fig. 7**). Analysis of the diradylglycerol trimethylsilyl ether derivative by mass spectrometry showed the anticipated molecular ion at *m/z* 665 and major fragments at *m/z* 409, 306, and 103 whose origins are consistent with the proposed structure (Fig. 6). The triradyl compound also exhibited a mass spectrum consistent with the proposed structure in which the molecular ion (*m/z* 831) and characteristic fragments at *m/z* 593 and 575 are prominent (Fig. 7). Accurate mass measurements of the molecular ions confirmed the elemental compositions.

At 75 μM, the activity with *sn*-2-monooleoylglycerol amide was 12.5% of that observed with *sn*-2-monooleoylglycerol; the di- and triradylglycerols formed 56 and 41%,

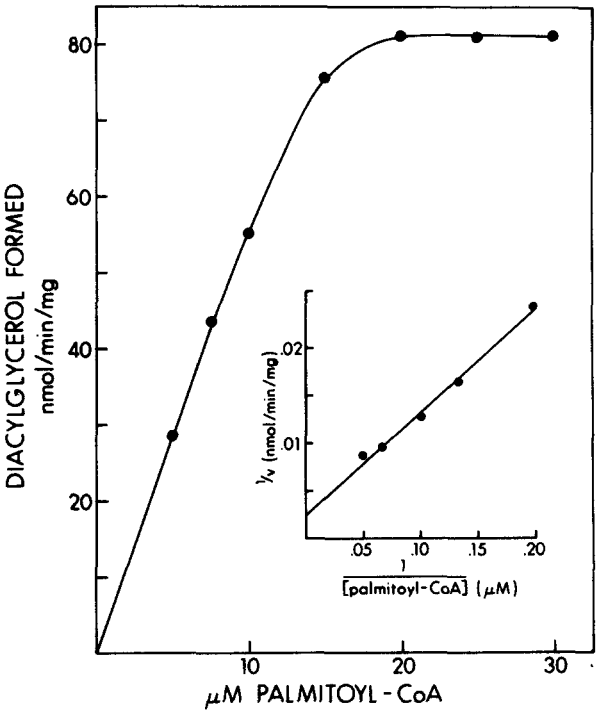

Fig. 4. Dependence of monoacylglycerol acyltransferase on palmitoyl-CoA. The activity was determined using 2 μg of hepatic microsomal protein from 18-day-old embryos. The insert shows the double-reciprocal plot according to the method of Lineweaver and Burk (19). The regression coefficient was 0.995.

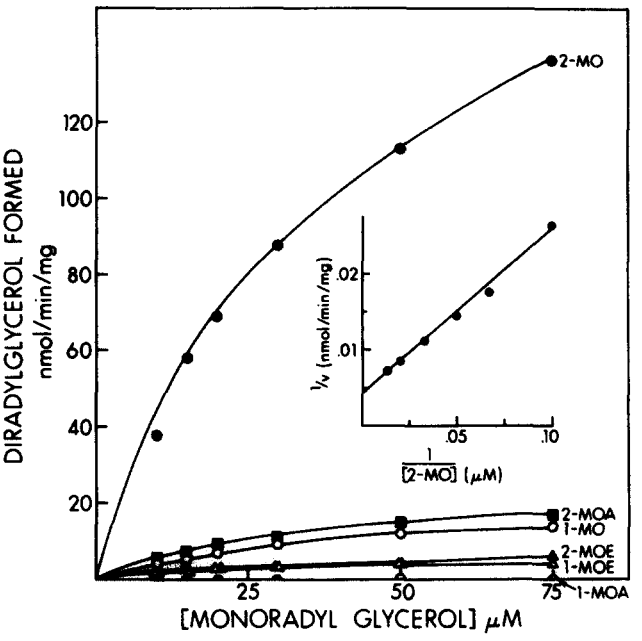

Fig. 5. Dependence of monoacylglycerol acyltransferase on *sn*-2-and 1(3)-monooleoylglycerols and their corresponding ether and amide analogs. Activities were determined using 2 μg of hepatic microsomal protein from 18-day-old embryos. The same microsomal preparation was used for all experiments. Monoradylglycerol solutions were prepared in acetone so that each desired concentration was achieved by adding 5 μl to the assay mixture. The insert shows a double-reciprocal plot of the dependence on *sn*-2-monooleoylglycerol according to the method of Lineweaver and Burk subjected to computer-assisted least squares analysis (19). The regression coefficient was 0.995. 1- and 1-MO = 1(3)- and 2-monooleoylglycerols; 1- and 2-MOE = 1(3)- and 2-monooleoylglycerol ethers; 1- and 2- MOA = 1(3)- and 2-monooleoylglycerol amides.

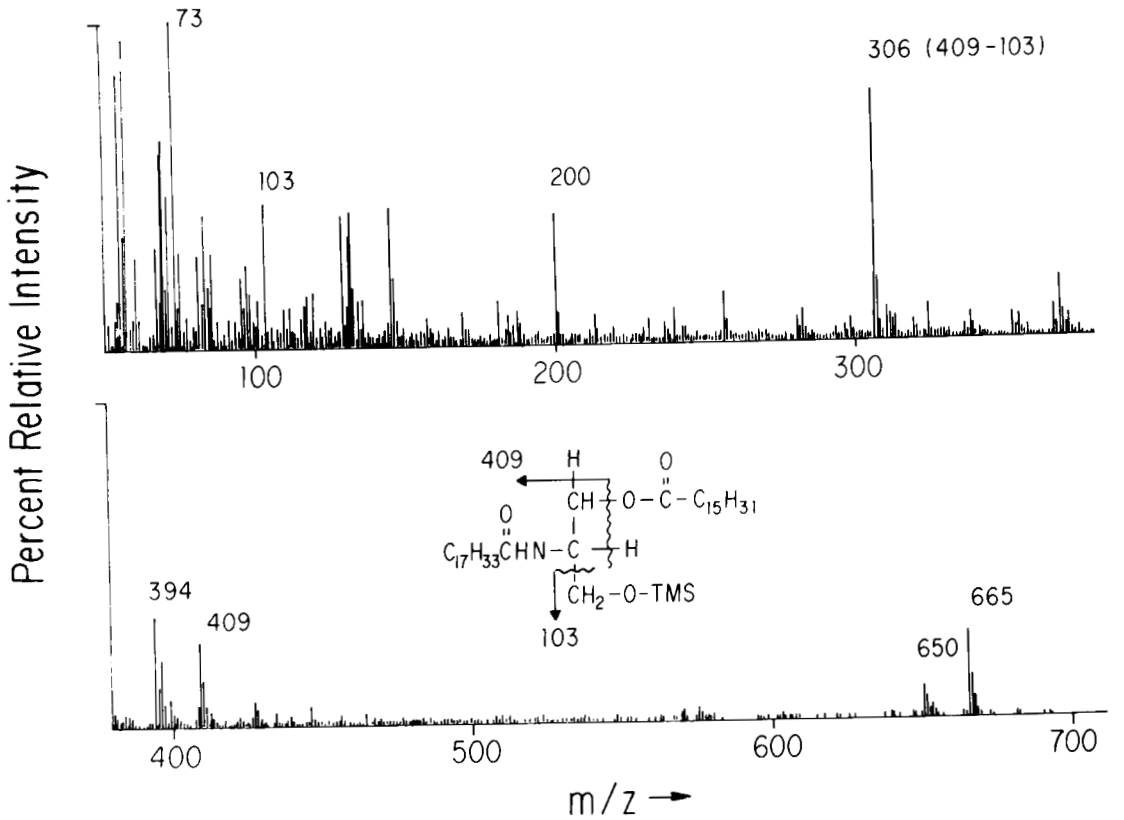

Fig. 6. Mass spectrum of the diradylglycerol obtained using *sn*-2-monooleoylglycerol amide and palmitoyl-CoA after derivitization with trimethylsilate (TMS). 1-Palmitoyl, 2-oleoylamide glycerol mass spectrum: *m/z* 665 ($M^{\cdot+}$), 650, 409, 396, 394, 306, 200, 103, 73. Accurate mass 665.5770 ($C_{40}H_{79}O_4$ NSi requires 665.5778, error 1.3 ppm).

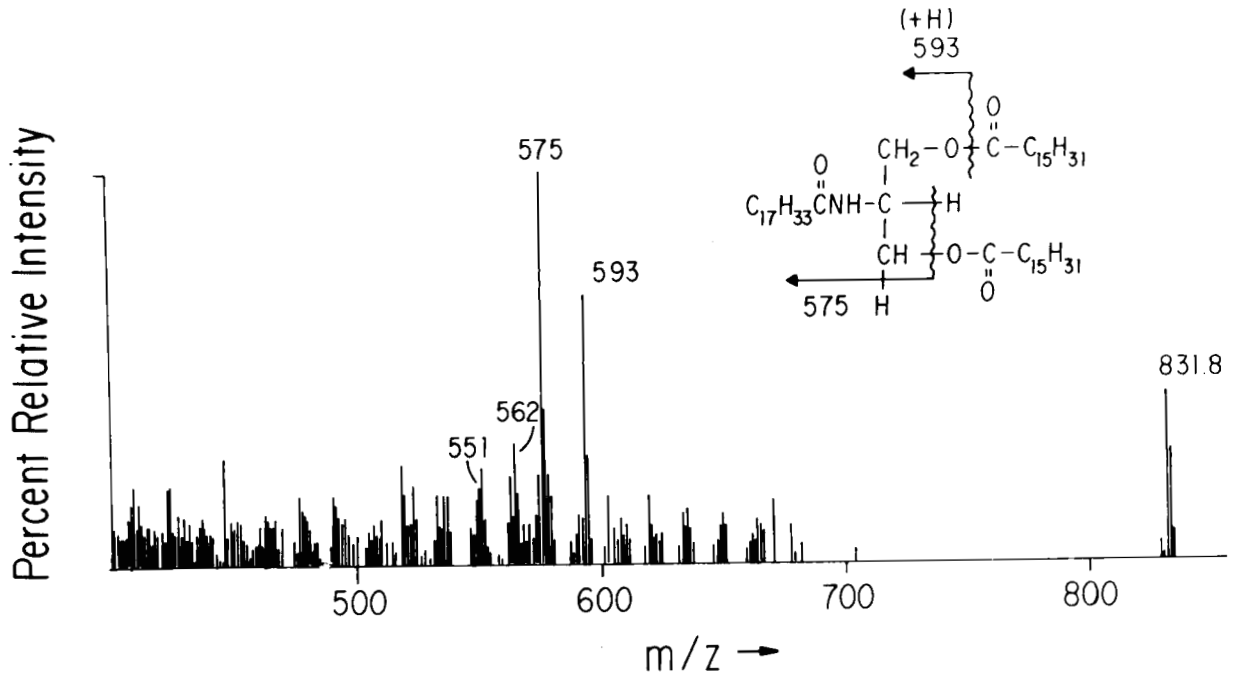

Fig. 7. Mass spectrum of the triradylglycerol obtained using *sn*-2-monooleoylglycerol amide and palmitoyl-CoA. 1,3-dipalmitoyl, 2-oleoylamide glycerol mass spectrum: 831 ($M^{\cdot+}$), 593, 575, 313, 256, 129. Accurate mass 831.7708 ($C_{53}H_{101}O_5N$ requires 831.7680, error 3.4 ppm).

TABLE 1. Monoradylglycerol dependencies

Substrate	Percent of Product[a] Formed[b]		Apparent K_m[c]	V_{max}[c]	Relative Activity[b]
	Diacylglycerol	Triacylglycerol			
			μM	nmol/min/mg	%
2-C18:1 glycerol	47	51	46.0	214	100
1-C18:1 glycerol[d]	8	89	41.2	20.5	9.1
2-C18:1 glycerol ether	22	74	29.6	6.8	4.4
1-C18:1 glycerol ether	20	78	16.4	5.0	2.9
2-C18:1 glycerol amide	53	42	30.6	22.8	12.5
1-C18:1 glycerol amide	19	36			< 0.1

[a]Acyl, alkyl, or amide analog.
[b]With 75 μM monoradylglycerol.
[c]Derived from a computer-assisted least squares analysis of data from Fig. 5 according to the method of Lineweaver and Burk (19). Regression coefficients were greater than 0.99 for the monoacylglycerols and greater than 0.98 for the ether and amide analogs. Each substrate was tested at least twice. Results varied by less than 10%.
[d]Activity may represent acylation of contaminating 2-C18:1 glycerol that has arisen via acyl-migration (20).

respectively, of the recovered ^3H label. The enhanced ability of the activity to acylate the 2-monooleoylglycerol amide in comparison to the corresponding ether suggests that the carbonyl group is important. The apparent K_m values were similar for all the acylglycerol analogs and seemed to bear no relationship to the corresponding V_{max} values (**Table 1**). With the 1- and 2-monooleoylglycerol ethers, about 70% of the product was recovered as triradylglycerol. In contrast, in microsomes from postnatal rat liver, only 6 to 9% of the product from the monoalkylglycerols is the triradylglycerol (2). This observation suggests that a diradylglycerol acyltransferase from chick liver can readily acylate 1-alkyl, 2-acylglycerols, perhaps via an activity similar to one recently described for HL-60 cells that acylates 1-alkyl, 2-acetyl(acyl)-*sn*-glycerols (21).

Tissue distribution

Monoacylglycerol acyltransferase specific activity was measured in total particulate fractions from tissues of 16-day chick embryos. In liver the mean activity ± SD was 41.5 ± 10.2 nmol/min per mg protein (n = 7). In two independent samples, average activities were 1.64 nmol/min per mg for intestine, 1.08 nmol/min per mg for gizzard, 0.90 nmol/min per mg for brain, 0.36 nmol/min per mg for heart. These activities were 25- to 115-fold lower than observed in liver.

Other enzyme activities of triacylglycerol synthesis

We compared the ontogeny of monoacylglycerol acyltransferase activity with that of other microsomal en-

TABLE 2. Activities of selected enzymes of triacylglycerol synthesis

	11- to 21-Day Embryos (n = 19)	11-Day Embryo[a] (n = 4)	Day-6 Chick[a] (n = 4)
DGAT[b]			
nmol/min/mg	24.2 ± 8.3		9.0 ± 1.4
nmol/min/liver		28.8 ± 12.5	4084 ± 667
MGAT[b]			
nmol/min/mg	34.5 ± 8.1		2.6 ± 1.3
nmol/min/liver		53.3 ± 14.2	1232 ± 689
Microsomal GPAT[b]			
nmol/min/mg	1.37 ± 0.5		0.64
nmol/min/liver		2.42	279
Mitochondrial GPAT[b]			
nmol/min/mg	0.51 ± 0.25		0.55
nmol/min/liver		1.3	245
Fatty acid CoA ligase			
nmol/min/mg	17.5 ± 5.8		18.1 ± 4.0
nmol/min/liver		34.8 ± 19.0	8180 ± 1048

[a]GPAT, n = 2. Except as noted, all activities are presented as means ± standard deviation.
[b]DGAT, diacylglycerol acyltransferase; MGAT, monoacylglycerol acyltransferase; GPAT, glycerol 3-P acyltransferase.

zymes of glycerolipid synthesis (**Table 2**). The specific activity of the committed step, the microsomal glycerol-P acyltransferase activity (NEM-sensitive), decreased during the time period examined. However, because the amount of total particulate protein increased 245-fold between the 11th day after fertilization and the 6th day after hatching, the microsomal glycerol-P acyltransferase activity per liver increased 115-fold during this time. The activity unique to triacylglycerol synthesis, the diacylglycerol acyltransferase, also decreased in specific activity after hatching, but there was a 142-fold increase in total activity per liver. Although the specific activities of fatty acid CoA ligase and mitochondrial glycerol-P acyltransferase did not change substantially, the total activities per liver increased 235- and 188-fold, respectively.

Effect of fasting on microsomal activities of glycerolipid synthesis

Since the activities of enzymes of fatty acid synthesis are altered substantially when dietary changes alter gene expression (8,9), we examined the activities of monoacylglycerol acyltransferase and selected enzymes of glycerolipid synthesis in liver microsomes from 3-day-old chicks that had been either fed or fasted since hatching. Little difference was apparent in the specific activitites of monoacylglycerol acyltransferase, microsomal or mitochondrial glycerol-P acyltransferase, or diacylglycerol acyltransferase in fed and fasted chicks (data not shown). Thus, none of the microsomal activities required for hepatic triacylglycerol biosynthesis appeared to be regulated by fasting or feeding.

DISCUSSION

This is the first description of hepatic monoacylglycerol acyltransferase activity from an avian species. Chick liver activity had characteristics similar to the monoacylglycerol acyltransferase from rat liver (2). Both activities had similar apparent K_m values for palmitoyl-CoA and *sn*-2-monooleoylglycerol and were inhibited similarly by $HgCl_2$, $MgCl_2$, KI, and $CaCl_2$, while remaining unaffected by a variety of other salts, chelators, and small molecule effectors. Unlike the monoacylglycerol acyltransferase activity from intestinal mucosa (4), the hepatic activities for both rat and chicken were highly specific for *sn*-2-monoacylglycerols and did not readily acylate 1(3)monooleoylglycerol and 1(3)- and *sn*-2-monooleoylglycerol ethers, although these substrates were acylated by the intestinal activity. Since both rat (Coleman, R. A., unpublished data) and chicken hepatic monoacylglycerol acyltransferase activities are able to acylate the *sn*-2-monooleoylglycerol amide but not the corresponding ether analog, the activity may require the presence of a carbonyl group.

High monoacylglycerol acyltransferase activity in the chick embryo was observed only in the liver. Although the intestinal activity was only 1.64 nmol/min per mg, the small size and fragility of the intestine from the day-16 embryo precluded separation of the mucosa from the underlying structures; thus, the value obtained in chick whole intestine may not be comparable to the activity of >100 nmol/min per mg observed in intestinal mucosa from the neonatal rat (2).

The ontogeny of the glycerolipid synthetic enzymes forms a marked contrast to that described for enzymes required for fatty acid synthesis (8,9). In fatty acid synthase, for example, enzyme activity, protein, and mRNA levels are very low in the chick embryo; after hatching, feeding increases transcription of the fatty acid synthase gene. Studies using cultured chick embryo hepatocytes indicate that thyroid hormone, glucagon, and insulin all play roles in the response of fatty acid synthase to feeding and fasting, suggesting that changes in the levels of these hormones are responsible for the dietary regulation that is seen in vivo. In contrast, the 90% decrease in monoacylglycerol acyltransferase specific activity after hatching appears to be independent of both diet and of the hormones implicated in the regulation of fatty acid synthase.

The source of the monoacylglycerol substrate is unclear. Egg yolk contains 5 to 6 g of lipid of which 71% is triacylglycerol and 22% is phospholipid (22). During the first 15 days of the 21-day incubation period, about 580 mg of lipid is transferred from the yolk to the embryo. The rate of lipid transfer increases markedly during the week before hatching and the internalized yolk sack continues to supply lipids to the chick through the week after hatching. The mechanism by which lipids are removed from the yolk and transferred to the embryo has not been well characterized molecularly. It is thought that triacylglycerol and phospholipids are taken up intact by the yolk sac membrane, which is an extension of the small intestine. There, triacylglycerol and phospholipids may be hydrolyzed, reesterified, and assembled into lipoproteins which enter the capillary system of the embryo (22). It is not known whether lipoprotein remnants or partially hydrolyzed triacylglycerol can enter the liver. In the rat, hepatic lipase is high neonatally (23,24). Since the lipase hydrolyzes monoacylglycerol (25), very little intact monoacylglycerol may enter the rat hepatocyte. Instead, the monoacylglycerol substrate in guinea pig and rat liver may arise via hydrolysis of intracellular triacylglycerol droplets (26,27). In contrast, the lipid that accumulates in developing chick liver is primarily cholesterol oleate (22) suggesting that the monoacylglycerol substrate in the chick may not arise from an intracellular source in the chick.

In the chicken, as in rat and guinea pig, the ontogeny of monoacylglycerol acyltransferase differed markedly from

that of other key microsomal enzymes of triacylglycerol synthesis (3,28). Unlike glycerol-P acyltransferase and diacylglycerol acyltransferase, monoacylglycerol acyltransferase specific activity declined precipitously after hatching to levels that were less than 10% of the activity found in embryo liver. In contrast, relatively little change was observed in the specific activities of fatty acid CoA ligase, microsomal or mitochondrial glycerol-P acyltransferase, or diacylglycerol acyltransferase activities.

These findings are qualitatively similar to previous observations of these microsomal activities in rat and guinea pig. In the guinea pig, monoacylglycerol acyltransferase activity decreased abruptly at birth (3) and in the rat, the activity decreased during the second postnatal week (2). The other glycerolipid synthetic enzyme activities, however, did not decrease at these times (3,28) suggesting that, in each species, monoacylglycerol acyltransferase and the activities of the glycerol-P pathway of triacylglycerol biosynthesis are regulated independently.

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