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Enzymes of Glycerolipid Synthesis in Small-Intestinal Mucosa of Foetal and Neonatal Guinea Pigs

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1. The activities of some enzymes of glycerolipid synthesis were measured in homogenates obtained from the intestinal scrapings of 62–66-day foetuses and 2- and 8-day-old guinea pigs. 2. The ratio of protein concentration/DNA concentration was significantly higher ($P < 0.001$) in homogenized tissue from the neonatal compared with the foetal guinea pigs. Enzyme activities were therefore expressed relative to both protein and to DNA. 3. The specific activities (relative to DNA) of palmitoyl-CoA synthetase, glycerol phosphate acyltransferase and phosphatidate phosphatase were higher in homogenized tissues from neonatal than in those from the foetal guinea pigs. These activities are probably involved more in cell proliferation than in lipid transport. 4. Monoacylglycerol acyltransferase activity is involved in the absorption and transport of triacylglycerol. Its activity was not significantly different in the foetal and neonatal guinea pigs when expressed relative to DNA but it was lower in the neonatal guinea pigs when expressed relative to protein. The entry of food into the intestine after birth is therefore not necessary for its activity.

The synthesis of triacylglycerols and of phospholipids in the small intestine is necessary for the transport of fat across the epithelial cells and for the synthesis of chylomicrons. We have investigated whether a number of the enzymes that are required for glycerolipid synthesis are present at birth in the mucosa of the small intestine of guinea pigs.

To do this, the activities of the following enzymes of the glycerol phosphate and of the 'monoacylglycerol' pathways of glycerolipid synthesis were measured as follows. (1) Palmitoyl-CoA synthetase (EC 6.2.1.3), which provides activated fatty acids for glycerolipid synthesis. Almost all of the activity of this enzyme is recovered in the microsomal fraction of mucosal homogenates of the small intestine of a number of species, although a proportion of the homogenate activity may be mitochondrial in origin (Brindley, 1974). (2) Glycerol phosphate acyltransferase (EC 2.3.1.15), which is the first enzyme of the glycerol phosphate pathway of triacylglycerol synthesis. This enzyme is recovered almost exclusively in the microsomal fraction of mucosal homogenates of the small intestine of a number of species (Brindley, 1974). (3) Phosphatidate phosphatase (EC 3.1.3.4), which is the subsequent enzyme in the glycerol phosphate pathway. This enzyme is unusual in that it is recovered predominantly in the soluble fraction of homogenates of the small intestine of a number of species (Brindley, 1974). (4) Monoacylglycerol acyltransferase (acylglycerol palmitoyltransferase, EC 2.3.1.22), which is the first enzyme involved in the stepwise acylation of 2-monoacylglycerol to triacylglycerol, i.e. the monoacylglycerol pathway. This is essentially a microsomal enzyme in homogenates of the small intestine (Brindley, 1974).

The total homogenates of the intestinal mucosa of foetal and neonatal guinea pigs were used to measure changes in the activities of these enzymes during development. This was dictated by the difficulties involved in fractionating the small amounts of intestinal scrapings that were obtained from these animals, and the dual subcellular distribution of the enzymes.

Experimental

Animals

Pregnant guinea pigs of the Dunkin–Hartley strain were obtained from the University of Nottingham Joint Animal Breeding Unit, Sutton Bonington, Leics., U.K. They were fed on Pilsbury's Diet TR2 (vitamin C-enriched) which was obtained from Heygate and Sons, Bugbrooke Mills, Northampton, U.K. The foetal guinea pigs were delivered by caesarian section. The neonatal guinea pigs were allowed to suckle freely.

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Materials

The chemicals and the enzymes used were purchased or prepared as described previously (Sánchez *et al.*, 1973; Mangiapane *et al.*, 1973; Short *et al.*, 1974).

Methods

Preparation of mucosal homogenates. These were prepared as described by Hübscher *et al.* (1965).

Enzyme assays. Each enzyme was assayed at several protein concentrations under optimum assay conditions. This ensured that the reaction rate was proportional to the enzyme concentration. The assays were usually done in duplicate.

(*a*) Palmitoyl-CoA synthetase. The method used was essentially that described by Sánchez *et al.* (1973). Each assay contained, in a volume of 0.25ml: 25mM-Tris buffer adjusted to pH7.4 with HCl; 5mM-dithiothreitol; 30μM-CoA; 8mM-ATP; 7.5mM-$MgCl_2$; 1.2mM-potassium palmitate; 1.5mg of fatty acid-poor bovine serum albumin; 3.75mM-(−)−[^{3}H]-carnitine (0.2μCi/μmol) and excess (about 620μg of protein) of carnitine palmitoyltransferase (EC 2.3.1.21). Up to 400μg of homogenate protein was used per assay and the incubation time was 6min at 37°C.

(*b*) Glycerol phosphate acyltransferase. The method used was essentially that described by Brindley (1973). Each assay contained, in a volume of 0.25ml: 25mM-Tris buffer, adjusted to pH7.4 with HCl; 5mM-dithiothreitol; 150μM-CoA; 0.6mM-palmitoyl-(−)-carnitine; 1.5mg of fatty acid-poor bovine serum albumin; an excess (about 220μg of protein) of carnitine palmitoyltransferase and 30mM-*sn*-[1,3-^{3}H]-glycerol 3-phosphate (0.5μCi/μmol). Up to 400μg of homogenate protein was used per assay. The incubation time was 6min at 37°C.

(*c*) Phosphatidate phosphatase. The method used was essentially that described by Brindley & Bowley (1975). The optimum assay conditions were as follows. Each assay contained, in a volume of 0.5ml: 20mM-potassium phosphate buffer, pH6.8; 25μl of [^{3}H]-phosphatidate which was bound to endoplasmic-reticulum membranes (Brindley & Bowley, 1975); 4.0mM-$MgCl_2$ and 3mg of fatty acid-poor bovine serum albumin. Up to 300μg of homogenate protein was used per assay. The incubation time was 15min at 37°C.

(*d*) Monoacylglycerol acyltransferase. The incubation system required for optimum activity of the enzyme and the isolation of the reaction products have been described by Short *et al.* (1974). 2-Hexadecylglycerol (1.44mM), which is an analogue of monoacylglycerol, was used as the acyl-acceptor and [9,10-^{3}H]palmitoyl-(−)-carnitine (100μM; 0.9μCi/μmol) was used to generate [9,10-^{3}H]-palmitoyl-CoA as the acyl-donor. Each assay contained up to 100μg of homogenate protein. The incubation time was 6min at 37°C.

Identification of lipids synthesized. Lipids were identified by co-chromatography with authentic samples when analysed by t.l.c. (Sánchez *et al.*, 1973).

Determination of protein and DNA. These were determined as described by Hübscher *et al.* (1965).

Determination of radioactivity. Radioactivity was measured by liquid-scintillation spectrometry (Sánchez *et al.*, 1973).

Statistical procedures. The results obtained were analysed statistically as described by Bradford-Hill (1967).

Results

The guinea pigs were divided into three groups. One group consisted of 62–66-day foetuses. This is just before term, since the normal period of gestation for these animals is 67–68 days. The crown–rump lengths of the foetuses (11.1±1.1cm; mean±1S.D. for 13 foetuses) is in the same order as other observed values of 12.0–12.3cm for this stage of gestation (D. G. Walker, personal communication). The second group consisted of 2-day-old neonatal animals, and the third group contained 8-day-old animals.

The weights of the foetal guinea pigs were not significantly different from those of the 2-day-old neonatal animals. The 8-day-old animals were significantly ($P<0.001$) heavier than the foetal and the 2-day-old animals (Table 1).

The concentration of protein relative to that of DNA phosphorus in the homogenates of intestinal

Table 1. *Body weights of foetal and of neonatal guinea pigs, and the ratio of protein/DNA phosphorus in mucosal homogenates of the small intestines of these animals*

The values are given as means±1S.D., and the numbers of animals are shown in parentheses. Significant differences ($P<0.001$) are indicated as follows: (a) between foetal and 2-day-old neonatal animals; (b) between foetal and 8-day-old neonatal animals; (c) between 2-day-old and 8-day-old neonatal animals.

	62–66-day foetuses	2-day-old animals	8-day-old animals
Body weight (g)	86±20 (13)	77±11 (11) (c)	135±14 (8) (b)
Protein (mg)/DNA P (μg) in mucosal homogenates	4.7±0.9 (13) (a)	6.9±1.4 (11)	6.5±0.8 (8) (b)

Table 2. *Activities of enzymes involved in glycerolipid synthesis in mucosal homogenates of the small intestines of foetal and neonatal guinea pigs*

The values are given as means ± 1 S.D., and the numbers of animals are shown in parentheses. Significant differences are indicated as follows: (a) between foetal and 2-day-old neonatal animals; (b) between foetal and 8-day-old neonatal animals; (c) between 2-day-old and 8-day-old neonatal animals The magnitude of the difference is indicated by $*P<0.05$, $**P<0.01$ and $***P<0.001$. The specific activities are expressed as nmol of substrate transformed/min related either to mg of homogenate protein or to μg of homogenate DNA phosphorus. The activity of monoacylglycerol acyltransferase was calculated from the rate of incorporation of palmitoyl-CoA into total neutral lipids and from the appropriate product composition shown in Table 3.

	Specific activity relative to protein			Specific activity relative to DNAP		
	62–66-day foetuses	2-day-old animals	8-day-old animals	62–66-day foetuses	2-day-old animals	8-day-old animals
Palmitoyl-CoA synthetase	17.5 ± 2.8 (13)	22.6 ± 5.7 (11)** (a)	25.8 ± 2.8 (8)*** (b)	80.6 ± 22.4 (13)	157.8 ± 47.2 (11)*** (a)	169.6 ± 28.0 (8)*** (b)
Glycerol phosphate acyltransferase	9.6 ± 1.8 (13)	10.0 ± 0.9 (9)	10.2 ± 2.3 (8)	46.6 ± 13.9 (13)	65.7 ± 8.9 (9)** (a)	68.1 ± 17.9 (8)** (b)
Phosphatidate phosphatase	2.4 ± 1.0 (13)	4.2 ± 1.0 (6)** (a)	2.8 ± 0.5 (7)** (c)	12.6 ± 6.4 (12)*** (a)	27.8 ± 7.8 (6)* (c)	18.6 ± 4.4 (7)* (b)
Monoacylglycerol acyltransferase	9.9 ± 1.9 (13)	7.7 ± 1.7 (10)** (a)	6.2 ± 1.6 (8)*** (b)	46.9 ± 14.3 (13)	50.2 ± 10.8 (10)	41.7 ± 12.7 (8)

mucosa obtained from the three groups is shown in Table 1. The homogenates of tissue from foetal animals contained significantly ($P<0.001$) less protein relative to DNA phosphorus than did the homogenates of tissue from the two neonatal groups of animals. There was no significant difference between the 2-day-old and the 8-day-old animals. These observations are important when the specific activities of the enzymes in the three groups of animals are compared (see Table 2). The expression of specific activities relative to DNA indicates the relative enzyme activity per cell and is therefore more easily interpreted.

Palmitoyl-CoA synthetase

The specific activity of this enzyme increased significantly from 62 to 66 days of gestation to both 2 days and 8 days *post partum* (Table 2). This increase is observed when the activity is expressed relative to protein or to DNA phosphorus. There is no significant increase in activity relative to protein or to DNA phosphorus between day 2 and day 8 *post partum*.

Glycerol phosphate acyltransferase

The specific activity of this enzyme is significantly higher in the 2-day-old and in the 8-day-old neonatal animals than in the foetal animals when the activity is expressed relative to DNA phosphorus (Table 2). There is no significant difference in specific activity relative to DNA phosphorus between 2-day-old and 8-day-old animals. The specific activities of glycerol-phosphate acyltransferase in the three groups of animals are not significantly different when they are expressed relative to homogenate protein.

Phosphatidate phosphatase

The specific activity of this enzyme increases significantly between 62–66 days of gestation and days 2 and 8 *post partum* when it is expressed relative to DNA phosphorus (Table 2). However, the specific activity decreases significantly between day 2 and day 8 *post partum* when it is expressed on either a protein or on a DNA phosphorus basis.

In addition, the proportion of acylglycerols synthesized by 8-day-old animals decreases and that of phosphatidate increases compared with the proportions synthesized by foetal and by 2-day-old neonatal animals (Table 3). The relative proportions of acylglycerols and of phosphatidate synthesized depends on the relative activities of phosphatidate phosphatase and of glycerol phosphate acyltransferase. The lower activity of phosphatidate phosphatase in the 8-day-old neonatal animals compared with the 2-day-old neonatal animals is consistent

Table 3. *Composition of the lipids synthesized during the assay of glycerol phosphate acyltransferase and monoacylglycerol acyltransferase in mucosal homogenates of small intestines of foetal and of neonatal guinea pigs*

The assay systems used are described under 'Methods'. The values are given as means ± 1 S.D. and the numbers of animals are shown in parentheses. Significant differences are indicated as follows: (a) between foetal and 2-day-old neonatal animals; (b) between foetal and 8-day-old neonatal animals; (c) between 2-day-old and 8-day-old neonatal animals. The magnitude of the difference is indicated by $*P<0.05$, $**P<0.02$, $***P<0.005$ and $****P<0.001$.

	Product composition (mol/100 mol)				
	Glycerol phosphate esterification			Monoacylglycerol esterification	
	Lysophosphatidate	Phosphatidate	Acylglycerols	Monoacyl-2-hexadecylglycerol	Diacyl-2-hexadecylglycerol
62–66-day foetuses	12±4 (13)	72±7 (13)	16±6 (13)	92±1 (13)*** (a)	8±1 (13)**** (a)
2-day-old neonatal animals	11±8 (11)	73±7 (11)* (c)	16±9 (11)** (c)	88±3 (10)*** (c)	12±3 (10)*** (c)
8-day-old neonatal animals	13±2 (8)	80±4 (8)** (b)	7±4 (8)**** (b)	84±2 (8)**** (b)	16±2 (8)**** (b)

with the lower proportion of acylglycerols synthesized by the latter group of animals (Table 3). This relationship was not observed when foetal guinea pigs were used. However, the activity of glycerol phosphate acyltransferase obtained with the foetal guinea pigs was about one-half of the equivalent activity obtained from the neonatal animals (Table 2).

Monoacylglycerol acyltransferase

This enzyme shows a significant decrease in specific activity related to homogenate protein when foetal animals are compared with 2-day-old and with 8-day-old animals (Table 2). There are no significant changes between the three groups of animals when the specific activity is related to DNA phosphorus.

The products obtained by the enzymic acylation of 2-hexadecylglycerol are shown in Table 3. The major product of the reaction is monoacyl-2-hexadecylglycerol, which is an analogue of diacylglycerol. This is also the major product of this assay when microsomal fractions prepared from homogenates of the small intestinal tract of adult guinea pigs are used (Short *et al.*, 1974). The proportion of diacylglycerol analogue formed decreases significantly, whereas that of the triacylglycerol analogue (i.e. diacyl-2-hexadecylglycerol) increases significantly from foetal to 2-day-old neonatal guinea pigs and from 2-day-old to 8-day-old neonatal guinea pigs (Table 3). This may indicate that diacylglycerol acyltransferase activity (EC 2.3.1.20) increases during these periods of development.

Relationships between the activities of the enzymes of glycerolipid synthesis

The activities (related to DNA) of the four enzymes of glycerolipid synthesis have been compared with the body weights of the guinea pigs by using linear regression analysis. There are significant correlations between the activities of palmitoyl-CoA synthetase ($P<0.05$) and of glycerol phosphate acyltransferase ($P<0.05$) when these are compared with the body weights using the results obtained from all of the foetal and neonatal guinea pigs. However, there is no significant correlation when the body weights of only the foetal guinea pigs are compared with the activities of these two enzymes. Nor is there any significant correlation between the activities of monoacylglycerol acyltransferase and of phosphatidate phosphatase when these are compared with the body weights of all the guinea pigs or with the body weights of the foetal group.

Significant correlations are observed between the activities of the four enzymes (related to DNA) when these are compared with one another. The correlations were: palmitoyl-CoA synthetase versus glycerol phosphate acyltransferase ($P<0.001$); palmitoyl-CoA synthetase versus monoacylglycerol acyltransferase ($P<0.05$); palmitoyl-CoA synthetase versus phosphatidate phosphatase ($P<0.001$); monoacylglycerol acyltransferase versus glycerol phosphate acyltransferase ($P<0.05$); monoacylglycerol acyltransferase versus phosphatidate phosphatase ($P<0.05$); glycerol phosphate acyltransferase versus phosphatidate phosphatase ($P<0.05$). However, the intercept of the regression line was significantly different from zero ($P<0.05$ or $P<0.01$) for all pairs of enzymes except when comparing acyl-CoA synthetase and phosphatidate phosphohydrolase. A positive correlation was also found between the activities of palmitoyl-CoA synthetase and glycerol phosphate acyltransferase in the microsomal fractions of intestinal mucosa obtained from adult guinea pig (Brindley, 1973). In this work the intercept was also significantly different from zero.

Discussion

There are many reports that the composition of the diet alters the activities of a number of enzymes involved in glycerolipid synthesis in the small

intestine of adult animals. Starvation depresses the activities of microsomal palmitoyl-CoA synthetase and monoacylglycerol acyltransferase (McManus & Isselbacher, 1970; Tandon *et al.*, 1972; Powell & McElveen, 1974). Feeding a fat-enriched diet increases the activities of monoacylglycerol acyltransferase (Rodgers & Singh, 1972; Singh *et al.*, 1972) and of diacylglycerol acyltransferase (EC 2.3.1.20), lysolecithin acyltransferase (EC 2.3.1.23) and choline phosphotransferase (EC 2.7.8.2) (Mansbach, 1975). There is also evidence that feeding a diet enriched with fat increases the activity of phosphatidate phosphatase in the intestine of the adult frog (Sarzala & Wlodawer, 1969). In contrast with these reports, the experiments of Rao & Abraham (1974) indicate that starving or feeding with a fat-enriched diet does not alter the specific activities of microsomal monoacylglycerol acyltransferase in the small intestine of adult rats or hamsters.

Experiments with rats have shown that the activities of palmitoyl-CoA synthetase and monoacylglycerol acyltransferase were higher in the small intestinal mucosa of newborn compared with foetal and adult animals (Eisen *et al.*, 1973; Holtzapple *et al.*, 1974, 1975). The experiments reported here investigated whether the activities of enzymes required for intestinal lipid synthesis were increased by the availability of substrates obtained from the diet of the young guinea pigs.

We have found no significant increase in the specific activity (relative to DNA) or monoacylglycerol acyltransferase in the 2-day-old and in the 8-day-old guinea pigs compared with the pre-partum animals. In fact, the specific activity relative to protein decreased in the neonatal animals. Monoacylglycerol acyltransferase is involved in the re-esterification of partial acylglycerols which are derived from the diet and accounts for the major transport of acylglycerols across the enterocytes [see Brindley (1974) for review]. The specific activities (related to DNA) of palmitoyl-CoA synthetase, glycerol phosphate acyltransferase and phosphatidate phosphatase increased 2 days after birth compared with the foetal guinea pigs. Palmitoyl-CoA synthetase is needed to generate activated fatty acids for both the monoacylgycerol and glycerol phosphate pathways, whereas glycerolphosphate acyltransferase and phosphatidate phosphatase are concerned with the synthesis of glycerol lipids *de novo*. It is likely that much of the activity of the enzymes of the glycerol phosphate pathway are concerned with cell proliferation in the intestine rather than with the direct transport of dietary fat (Mansbach, 1975).

The diet may well affect the activities of the enzymes responsible for fat transport in the small intestine. However, the experiments reported here indicate that the enzymes of the monoacylglycerol pathway are active in the intestine before suckling takes place. These results agree with those of J. M. Johnston (personal communication). He showed that the activity of monoacylglycerol acyltransferase (relative to protein) is markedly increased in the small intestinal mucosa of rabbits after 27–30 days of gestation.

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