Triglyceride Synthesis by the Small-Intestinal Epithelium of the Pig, Sheep and Chicken

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1. A comparative study was made of triglyceride synthesis by the intestinal epithelium of pigs, sheep and chickens. In pig and chicken tissue both the glycerol 3-phosphate and the monoglyceride pathway of triglyceride synthesis were operative, but the former pathway predominated in sheep tissue. 2. The fatty acid specificity of the glycerol 3-phosphate pathway was studied in pig and sheep total-homogenate preparations. Maximum incorporation was obtained with myristic acid and palmitic acid under optimum conditions for each fatty acid. Lauric acid, myristic acid, oleic acid, linoleic acid and linolenic acid were inhibitory at concentrations above their optimum, but octanoic acid, decanoic acid, palmitic acid and stearic acid did not show this effect. 3. Subcellular fractionation located the glycerol 3-phosphate and monoglyceride pathways of triglyceride synthesis in the microsomes in all instances. Phosphatidate phosphohydrolase was associated with both the microsomes and the particle-free supernatant. 4. Glycerol 1-mono-oleate was incorporated into triglycerides to a greater extent than glycerol 1-mono-palmitate or glycerol 1-monostearate by microsomal preparations from pig and chicken. 5. A lipase specific for monoglycerides was detected in the particle-free supernatant of all the species examined.

The digestion and absorption of fats in non-ruminants occurs largely in the small intestine. There is some evidence for the absorption of particulate fat in certain species (Wilson, 1962; Senior, 1964), but in man (Borgström, Lundh & Hofmann, 1963) and pig (Freeman, Annison, Noakes & Hill, 1967) there is considerable evidence that fat is absorbed largely as micelles rich in monoglycerides and fatty acids. Recent studies have shown that micelles of similar composition have a comparable role in fat absorption in the chicken (C. P. Freeman, unpublished work), although there is no uptake of fat by the intestinal lymphatics in this species (Noyan, Lossow, Brot & Chaikoff, 1964).

In ruminants, dietary fat is rapidly hydrolysed in the rumen, and the fat entering the duodenum contains a high proportion of free fatty acid and little glyceride (Bath & Hill, 1967). Micelle fat absorption may well be quantitatively important in ruminants, however, with lysolecithin taking over the role of monoglycerides in micelle formation (Bath & Hill, 1967; Garton, 1967; Leat & Harrison, 1967; Lennox, Lough & Garton, 1968).

The differences in fat digestion, absorption and transport between ruminants and non-ruminants made it of interest to compare triglyceride synthesis

in the intestinal epithelium of pig, chicken and sheep, particularly as these species have not been examined in detail before. In other species, such as the cat (Brindley & Hübscher, 1965, 1966) and hamster (Johnston, 1959; Brown & Johnston, 1964), two pathways of triglyceride synthesis have been delineated, based on glycerol 3-phosphate or monoglyceride as acceptors of activated fatty acids. In the present studies, both pathways were demonstrated in the pig and chicken and all the necessary enzymes for the two pathways were located in the microsomes. Phosphatidate phosphohydrolase (EC 3.1.3.4), recently detected in the particle-free supernatant of cat (Smith, Sedgwick, Brindley & Hübscher, 1967) and hamster intestinal epithelium (Johnston, Rao, Lowe & Schwarz, 1967), and in certain particulate fractions of cat and rat tissue (Smith *et al.* 1967), was found to be associated largely with the microsomal fraction of pig and sheep tissue. In microsomal preparations of sheep intestinal epithelium, only the glycerol 3-phosphate pathway could be detected unequivocally: 1-monoglycerides showed only a slight capacity to accept activated fatty acids under conditions similar to those used for demonstrating this pathway in pig and chicken tissues. Leat & Cunningham (1968) reported the direct incorporation of monoglycerides

into triglycerides by the sheep small intestine *in vivo* and by segments of sheep small intestine *in vitro*.

Examination of the specificity of the glycerol 3-phosphate pathway in the sheep (ruminant) and pig (non-ruminant) showed that there was little difference between the species in their capacity to activate and incorporate fatty acids into triglycerides.

MATERIALS AND METHODS

Experimental animals. Newly weaned pigs (age 3–4 weeks) were killed by an intravenous injection of sodium barbiturate, and a length (100 cm.) of small intestine (located 75–175 cm. from the pyloric sphincter) was immediately removed, washed with 0.9% (w/v) NaCl, everted with a glass rod and then further washed with 0.9% NaCl. Sheep (rams, age 2–3 yr.) and chicken (hens, age 12–18 months) tissues were obtained by similar procedures, except that the lengths of small intestine used were located 100–300 cm. and 0–75 cm. from the pyloric sphincter respectively.

When the distribution of triglyceride synthetase activity along the small intestine was examined, the whole tract was removed as quickly as possible, and the chicken, pig and sheep small intestines were cut into 25 cm., 50 cm. and 100 cm. lengths respectively. The tissues were then washed as before.

Preparation of homogenates of small-intestinal mucosa. The general procedure of Hübcher, West & Brindley (1965) was used for the tissues from all three species. The everted intestine was placed on a glass plate and the mucosa removed by gentle scraping with a glass slide held at 45° to the epithelium. The mucosal scrapings were collected in foil and weighed. Portions (1 g.) were suspended in 10 ml. of 0.25 M-sucrose–5 mM-EDTA, and homogenized with six strokes in a Teflon-glass homogenizer with the plunger rotating at 700 rev./min. (plunger clearance 0.012 in.). The homogenate was centrifuged at low speed for 1 min. The supernatant was set aside and the sedimented unhomogenized material was homogenized again with three strokes in an equal volume of 0.25 M-sucrose–5 mM-EDTA. The homogenate was centrifuged again at low speed and the resultant pooled supernatant constituted the total homogenate. The entire procedure was carried out at 4°. In later experiments 3 mM-tris-HCl buffer, pH 7.4, was included in the homogenization medium and was found to increase the stability of the enzymes.

Fractionation of the mucosal homogenate. The homogenate was centrifuged for 10 min. at 1500g to remove the brush borders (Miller & Crane, 1961). Care was taken to avoid rupture of the brush borders during homogenization to prevent contamination of the mitochondrial and microsomal fractions with brush-border fragments. The supernatant was centrifuged for 15 min. at 12000g and for 60 min. at 105000g to isolate the mitochondria and microsomes respectively. Washing the microsomal fraction resulted in a marked loss of activity, measured in terms of capacity to incorporate fatty acid into triglyceride, and so the unwashed material was used in all the studies except where otherwise stated. The particulate subcellular fractions were suspended in 0.25 M-sucrose–5 mM-EDTA in an all-glass homogenizer to give protein concentration of approx. 5 mg./ml. The

final supernatant constituted the particle-free supernatant. Examination of the mitochondrial and microsomal fractions with the electron microscope showed that the fractions were not completely pure, since a number of membranous structures were observed in the mitochondrial preparation, but that the microsomal fraction was largely free of mitochondria.

The enzymes succinate dehydrogenase and glucose 6-phosphatase are usually associated with mitochondria and microsomes respectively (Hübcher *et al.* 1965). In the present work the mitochondria contained 95% of the succinate dehydrogenase activity, and the microsomes 56% of the glucose 6-phosphatase activity.

Assay of triglyceride synthesis. Triglyceride synthesis was determined by the extent of incorporation of radioactive fatty acid into triglycerides in the presence of either glycerol 3-phosphate or monoglycerides. The assay system (final volume 3 ml.) contained: 250 μ moles of potassium phosphate buffer, pH 7.4, 3 μ moles of fatty acid, 20 μ moles of ATP, 0.1 μ mole of CoA, 20 μ moles of MgCl₂, 30 μ moles of glycerol 3-phosphate or 2–5 μ moles of monoglyceride, and approx. 5 mg. of protein. In early experiments GSH (8 μ moles) and KF (20 μ moles) were included in the incubation medium, but these components were found to have no effects on the activity of tissue homogenates, and to inhibit triglyceride synthesis by microsomal fractions substantially. The reaction was started by adding the enzyme preparation. Incubations were usually carried out at 37° for 60 min., after which the reaction was stopped by the addition of 7.5 ml. of methanol followed by 6.5 ml. of water and 13.0 ml. of chloroform to extract lipid (Bligh & Dyer, 1959). The upper aqueous layer and interfacial protein were removed by suction and re-extracted with 10 ml. of chloroform. The chloroform extracts were pooled and evaporated to dryness under a stream of N₂ at 40°. The recovery of radioactivity as lipid was about 98%.

Separation of lipids. The extracted lipid was dissolved in a known volume of chloroform-methanol (2:1, v/v), a suitable sample was removed for thin-layer chromatography, 0.02 ml. of olive oil was added to provide carrier lipid, and the whole mixture was transferred to a glass plate coated with silicic acid (0.4 mm. thick). The plate was developed in toluene-diethyl ether-ethanol-acetic acid (250:240:10:1, by vol.) and the bands corresponding to triglyceride, diglyceride, free fatty acid and monoglyceride, and the material at the origin (phospholipid), were scraped off and transferred directly to radioactivity-counting vials. The bands were located by using marker spots, and by spraying with aq. 0.1% Ultraphor followed by location under a u.v. lamp. The radioactivities of the lipid bands were determined in 15 ml. of toluene containing 3.8% of low-density silica (Cab-O-Sil; Packard Instrument Co., La Grange, Ill., U.S.A.) and 0.4% of 2,5-diphenyloxazole in a Packard Tri-Carb liquid-scintillation spectrometer (series 4000). Phosphatidic acid was determined as described above, except that the plate was developed in chloroform-methanol-aq. 30% (v/v) methylamine (65:25:8, by vol.) by the method of Kuhn (1967).

Protein determination. Proteins (1–10 mg.) were precipitated with 2 ml. of aq. 8% (w/v) HClO₄. The sediment was collected by centrifugation, washed with ethanol-diethyl ether (1:1, v/v) to remove lipids, and dissolved in 1.0 ml. of aq. 3% (w/v) NaOH. Fresh biuret reagent (5 ml.) prepared by the method of Aldridge & Johnson (1959) was added, the

tubes were shaken for 10 min., and the suspension was filtered. E_{555} of the filtrate was read against a reagent blank. A calibration curve was prepared with crystalline bovine albumin.

Assay of succinate dehydrogenase (EC 1.3.99.1). The activity was measured by the extent of reduction of 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride to formazan (Pennington, 1961).

Assay of glucose 6-phosphatase (EC 3.1.3.9). The activity was determined by the production of P_i from glucose 6-phosphate (Hübscher & West, 1965).

Materials. Octanoic acid, decanoic acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A. The corresponding $1\text{-}^{14}\text{C}$ -labelled fatty acids were purchased from The Radiochemical Centre, Amersham, Bucks. DL-Glycerol 3-phosphate, ATP, CoA, GSH, glycerol 1-mono-oleate, glycerol 1-monopalmitate, glycerol 1-monostearate, glycerol trioleate and glycerol tristearate were supplied by Sigma Chemical Co.

Radioactive glycerol 1-mono-oleate and glycerol 1-monostearate were obtained by the action of pancreatic lipase on glycerol tri[$1\text{-}^{14}\text{C}$]oleate and glycerol tri[$1\text{-}^{14}\text{C}$]stearate as described by Mattson & Volpenheim (1961). The mono-glycerides were purified by thin-layer chromatography with the solvent system toluene-diethyl ether-ethanol-acetic acid (250:240:10:1, by vol.) and checked for the presence of other homologues by transmethylation followed by gas-liquid chromatography of the fatty acid methyl esters. The products were 98% pure.

Aqueous emulsions of the glycerides were prepared by the method of Clark & Hübscher (1961) by homogenization in 0.4% (w/v) Tween 80 (Honeywell and Stein Ltd., London, W. 1). Aqueous suspensions of the radioactive fatty acids were prepared by adding the requisite amount of an ethereal solution of the acids to a 50% excess of 12M-N-KOH and heating the solution to 50°. The short-chain and unsaturated fatty acids were heated to 35°. The solutions of the fatty acids had a specific radioactivity of 0.08 $\mu\text{C}/\mu\text{mole}$.

RESULTS

Distribution of synthetase activity. The homogenates from each length of pig, sheep and chicken small intestine were examined for their capacity to effect triglyceride synthesis from added fatty acids and glycerol 3-phosphate. The specific activities (μmoles of fatty acid incorporated/mg. of protein/hr.) of each length of intestine were in the order chicken > sheep > pig (Fig. 1). In all cases, maximum activity was found in the duodenum and jejunum, and as the distance from the duodenum increased the activity decreased.

Glycerol 3-phosphate pathway of triglyceride synthesis. The optimum conditions for triglyceride synthesis from glycerol 3-phosphate and [$1\text{-}^{14}\text{C}$]palmitate were determined by the extent of incorporation of radioactivity into triglycerides in the total homogenate of pig intestinal epithelium. When ATP and CoA were the only cofactors present fatty acid incorporation was low, but the addition of 20 μmoles of Mg^{2+} /3 ml. stimulated the synthesis

Table 1. *Effects of various additions on triglyceride synthesis by the total homogenate of pig intestinal epithelium*

ATP (20 μmoles), CoA (0.1 μmole), Mg^{2+} (20 μmoles), GSH (8 μmoles) and KF (20 μmoles) were added, as indicated, to the incubation medium (see the text) with the homogenate (4.5 mg. of protein).

Additions to incubation medium	Incorporation of [$1\text{-}^{14}\text{C}$]palmitate ($\mu\text{moles/hr./mg. of protein}$)
ATP, CoA	23
ATP, CoA, Mg^{2+}	184
ATP, CoA, Mg^{2+} , GSH	190
ATP, CoA, Mg^{2+} , GSH, KF	178

Table 2. *Triglyceride synthesis from glycerol 3-phosphate and [$1\text{-}^{14}\text{C}$]palmitate by subcellular fractions of pig, sheep and chicken intestinal epithelium*

For experimental details, see the text. Washed microsomes were used. PFS, Particle-free supernatant.

Fraction	Incorporation of [$1\text{-}^{14}\text{C}$]palmitate ($\mu\text{moles/hr./mg. of protein}$)		
	Pig	Sheep	Chicken
Homogenate	110	220	150
Mitochondria	135	15	137
Microsomes	222	103	153
PFS	0	2	13
Microsomes + PFS	1117	333	630

of triglycerides ninefold (Table 1). Further fortification of the incubation medium with GSH (8 μmoles) and potassium fluoride (20 μmoles) had no effect on glyceride synthesis.

The effects of varying the concentrations of ATP, CoA, glycerol 3-phosphate, palmitic acid and tissue protein at constant concentrations of Mg^{2+} were examined in homogenates of pig, sheep and chicken epithelial tissue (Fig. 2). The cofactor requirements were closely similar for each species. The optimum pH of the incubation medium was 7.4 (Fig. 3) and this was used in all subsequent experiments.

The subcellular distribution of the enzymes effecting glyceride synthesis by the glycerol 3-phosphate pathway in the intestinal-epithelium tissue of the three species is shown in Table 2. The activity associated with the microsomal fraction was markedly raised in the presence of the particle-free supernatant (2.0 ml.) (Table 2).

Role of the particle-free supernatant, and of GSH and potassium fluoride. The interrelationships between the microsomal and particle-free-supernatant fractions were examined in more detail in

Fig. 1. Distribution of the glycerol 3-phosphate pathway of triglyceride synthesis along the small intestines of (a) pig, (b) sheep and (c) chicken.

Fig. 2. Effects of the concentration of glycerol 3-phosphate (a), ATP (b), CoA (c) and palmitate (d) on triglyceride synthesis from glycerol 3-phosphate and [1-¹⁴C]palmitate by homogenates (5 mg. of protein) of pig (●), sheep (○) and chicken (Δ) intestinal epithelium, the concentrations of other components being optimum in each case.

pig and sheep epithelial tissues. A study of the cofactor requirements of the microsomal preparations showed that triglyceride synthesis occurred in a medium containing only ATP, CoA and Mg^{2+} , but

addition of GSH and potassium fluoride, frequently included in similar incubation mixtures (Brindley & Hübscher, 1965; Johnston *et al.* 1967) markedly decreased the yield of triglyceride. Addition of

particle-free supernatant (2.0 ml.) increased the rate of triglyceride synthesis in the presence and absence of GSH and potassium fluoride (Table 3).

More information on the effects of particle-free supernatant on triglyceride synthesis by the microsomal preparations was obtained by measuring the incorporation of [1-¹⁴C]palmitate into phosphatidic acid and triglyceride. The effects of varying the concentration of Mg²⁺ on the concentration of phosphatidic acid and triglyceride with microsomes and microsomes plus particle-free supernatant (2.0 ml.) are shown in Fig. 4. In both cases, triglyceride synthesis was stimulated by Mg²⁺ and an optimum concentration of 20 μmoles/3 ml. for the microsomes and 40 μmoles/3 ml. for microsomes plus particle-free supernatant (2.0 ml.)

Fig. 3. Effect of pH on triglyceride synthesis from [1-¹⁴C]-palmitate and glycerol 3-phosphate by the homogenate of pig intestinal epithelium.

was observed. The additional Mg²⁺ required when particle-free supernatant was added was almost certainly due to the binding of Mg²⁺ by EDTA in the particle-free supernatant, since increasing quantities of particle-free supernatant progressively decreased triglyceride synthesis by the pig microsomal preparation (Fig. 5). Mg²⁺ (40 μmoles/3 ml.) was used in all incubations containing 2 ml. of particle-free supernatant.

The fall of phosphatidic acid concentration on the addition of particle-free supernatant (Fig. 4) was consistent with the presence of phosphatidate phosphohydrolase in the particle-free supernatant, as suggested by earlier work with other species (Johnston *et al.* 1967; Smith *et al.* 1967). The time-course of incorporation of [1-¹⁴C]palmitate into phosphatidic acid and triglyceride in the presence and absence of particle-free supernatant is shown in Fig. 6. The accumulation of phosphatidic acid in the absence of particle-free supernatant was further evidence for the presence of a soluble phosphatidate phosphohydrolase in the particle-free supernatant, although the synthesis of triglycerides in the washed microsomal particles clearly indicated the presence of particulate-bound phosphatidate phosphohydrolase.

Monoglyceride pathway of triglyceride synthesis. When total homogenates of pig, sheep and chicken intestinal mucosa were incubated with [1-¹⁴C]-palmitate under the conditions used above, but with glycerol 1-mono-oleate replacing glycerol 3-phosphate as fatty acid acceptor, some triglyceride synthesis occurred with all tissues (Table 4). When the subcellular fractions of each tissue were examined for monoglyceride-pathway activity, pig tissue proved as active as chicken tissue, but sheep tissue showed only slight activity (Table 4). Most of the activity of pig and chicken tissue was associated with the microsomal fractions. The decrease of activity of these fractions on the

Table 3. Effects of particle-free supernatant, GSH and potassium fluoride on triglyceride synthesis from glycerol 3-phosphate and [1-¹⁴C]palmitate by the microsomal fractions of pig and sheep intestinal epithelium

		Components omitted	Incorporation of
		from incubation	[1- ¹⁴ C]palmitate
Species	Tissue fractions	medium	(mμmoles/hr./mg. of protein)
Pig	Microsomes	—	42
	Microsomes	GSH, KF	295
	Microsomes + PFS	—	162
	Microsomes + PFS	GSH, KF	342
Sheep	Microsomes	—	102
	Microsomes	GSH, KF	249
	Microsomes + PFS	—	333
	Microsomes + PFS	GSH, KF	365

Fig. 4. Effect of Mg^{2+} concentration on triglyceride (●) and phosphatidic acid (○) synthesis from glycerol 3-phosphate and $[1-^{14}C]$ palmitate by microsomal preparations of sheep [(a) and (b)] and pig [(c) and (d)] intestinal epithelium with [(b) and (d)] and without [(a) and (c)] added particle-free supernatant (PFS) (2.0 ml.).

Fig. 5. Effect of the particle-free supernatant on triglyceride (●) and phosphatidic acid (○) synthesis from $[1-^{14}C]$ -palmitate and glycerol 3-phosphate by a microsomal preparation of pig epithelial tissue. For concentrations of other components, see the text.

addition of the particle-free supernatant suggested the possible presence of monoglyceride lipase activity. Examination of the total homogenate of pig tissue and of the particle-free supernatant confirmed this hypothesis.

Glycerol 1-mono-oleate was a more effective fatty acid acceptor than glycerol 1-monostearate or glycerol 1-monopalmitate when examined at identical concentrations with microsomal preparations from pig and chicken intestinal epithelium (Table 5).

Monoglyceride lipase activity. The apparent lack of monoglyceride activity in the pig tissue homogenate and the positive result obtained with the microsomal fraction suggested the presence of a monoglyceride lipase. This possibility was checked by incubating the total homogenate with glycerol tri $[1-^{14}C]$ oleate and glycerol mono $[1-^{14}C]$ oleate. There was no release of labelled oleate from the triglyceride, but extensive splitting of the monoglyceride was observed. The monoglyceride lipase was located mainly in the particle-free supernatant (Table 6). Some activity was also observed in the

Fig. 6. Effect of added particle-free supernatant on triglyceride (●) and phosphatidic acid (○) synthesis from glycerol 3-phosphate and [1-¹⁴C]palmitate by microsomal preparations of sheep [(a) and (b)] and pig [(c) and (d)] intestinal epithelium with [(b) and (d)] and without [(a) and (c)] added particle-free supernatant (PFS) (2.0 ml.).

Table 4. Triglyceride synthesis by the monoglyceride pathway by subcellular fractions of pig, sheep and chicken intestinal epithelium incubated with glycerol 1-mono-oleate and [1-¹⁴C]palmitate

For experimental details, see the text. PFS, Particle-free supernatant.

Fraction	Incorporation of [1- ¹⁴ C]palmitate (mμmoles/hr./mg. of protein)		
	Pig	Sheep	Chicken
Homogenate	—	38	90
Mitochondria	32	—	270
Microsomes	326	6	340
Microsomes + PFS	30	4	90
PFS	—	—	—

particle-free supernatant of chicken tissue. The transfer of radioactivity into triglyceride by sheep tissue homogenate presumably reflects the utilization of glycerol 3-phosphate produced from the

glycerol liberated from the monoglyceride (Table 6). The presence of glycerokinase in this tissue was demonstrated in subsequent experiments (R. Bickerstaffe, unpublished work).

Specificity of fatty acid incorporation by the glycerol 3-phosphate pathway. The influence of chain length, degree of unsaturation of the eighteen-carbon fatty acids, and concentration on the incorporation of fatty acids into triglycerides was examined in the total tissue homogenates from pigs and sheep. The incubation conditions were those known to be optimum for palmitate incorporation. Incorporation of fatty acids of chain length < octanoic acid was negligible in both cases. All of the fatty acids examined showed an optimum concentration for incorporation, but lauric acid, myristic acid, oleic acid, linoleic acid and linolenic acid were inhibitory at concentrations higher than optimum (Fig. 7). The effect of fatty acid chain length and degree of unsaturation on the extent of incorporation into triglycerides at optimum concentrations for triglyceride synthesis are shown in Fig. 8. Pig and sheep tissue gave similar results.

Table 5. *Triglyceride synthesis by microsomal preparations of pig, sheep and chicken tissue from glycerol 1-monopalmitate, glycerol 1-mono-oleate and glycerol 1-monostearate*

For experimental details, see the text.

Species	Monoglyceride acceptor ...	Incorporation of [1- ¹⁴ C]palmitate (μmoles/hr./mg. of protein)		
		Glycerol 1-monopalmitate	Glycerol 1-mono-oleate	Glycerol 1-monostearate
Pig		126	326	26
Sheep		2	13	15
Chicken		137	340	28

Table 6. *Lipase activity of subcellular fractions of pig, sheep and chicken intestinal epithelium assayed by incubation with glycerol 1[1-¹⁴C]-mono-oleate*

The percentage distribution of radioactivity in the resultant phospholipids (PL), monoglycerides (MG), free fatty acids (FFA), diglycerides (DG) and triglycerides (TG) was measured. For details, see the text. PFS, Particle-free supernatant.

Species	Lipid fraction	Tissue fraction ...	Radioactivity in lipid fraction (% of the total for the tissue fraction)			
			Homogenate	Microsomes	Microsomes + PFS	PFS
Pig	PL		2	1	1	8
	MG		9	3	9	23
	FFA		51	2	71	61
	DG		9	9	4	1
	TG		29	85	15	7
Sheep	PL		2	5	1	2
	MG		27	38	14	49
	FFA		7	46	77	44
	DG		3	2	1	3
	TG		61	9	7	2
Chicken	PL		1	2	4	2
	MG		3	12	15	18
	FFA		9	33	33	75
	DG		16	35	15	—
	TG		71	18	33	5

DISCUSSION

A detailed comparison of the triglyceride synthetase activity of the epithelial tissues from the small intestines of pig, sheep and chicken has not been previously reported. There are two possible routes of triglyceride synthesis: the first utilizes glycerol 3-phosphate and has been demonstrated in the intestinal epithelium of cat (Brindley & Hübscher, 1966), hamster (Johnston, 1959), and rat (Dawson & Isselbacher, 1960). The second is the monoglyceride pathway first suggested by Clark & Hübscher (1960), and subsequently found in the cat (Brindley & Hübscher, 1965), rat (Senior & Isselbacher, 1962) and hamster (Brown & Johnston, 1964). The present studies demonstrated that with homogenates of all three tissues, triglycerides were

synthesized with glycerol 3-phosphate as the acyl acceptor of fatty acid, but only the chicken homogenate gave significant synthesis with 1-monoglyceride as the fatty acid acceptor. The conditions and cofactors necessary for the synthesis of triglycerides in homogenates were found to be similar to those required in identical studies with the intestinal epithelium of other species except that GSH and potassium fluoride were omitted (Johnston *et al.* 1967; Brindley & Hübscher, 1965).

Studies on the effects of varying the concentrations of different fatty acids on their incorporation into triglycerides with glycerol 3-phosphate as the acyl acceptor showed that the system was inhibited by certain fatty acids at concentrations above those required for optimum incorporation (Fig. 7). Brindley & Hübscher (1966) obtained similar results

Fig. 7. Effect of concentration of octanoic acid (8:0), decanoic acid (10:0), lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3) on their incorporation into triglycerides by the glycerol 3-phosphate pathway in the total homogenates of sheep [(a) and (b)] and pig [(c) and (d)] intestinal epithelium.

with cat mucosal tissue. The inhibition of esterification by certain long-chain unsaturated fatty acids may have no significance *in vivo*, where tissue concentrations of individual fatty acids are unlikely to reach inhibitory values. Rates of fatty acid activation or concentrations of glycerol 3-phosphate are more likely to regulate triglyceride synthesis (Kuhn, 1967; Brindley & Hübscher, 1966).

Subcellular fractionation revealed that in the pig, chicken and sheep epithelial cells, most of the triglyceride synthetase activity was located in the microsomes. There was some stimulation of microsomal triglyceride synthesis from glycerol 3-phosphate by the particle-free supernatant (Table 3). The stimulation has been reported, in the hamster, to be due to phosphatidate phosphohydrolase, which appears in the particle-free supernatant on subfractionation (Johnston *et al.* 1967). Smith *et al.* (1967) confirmed this for the cat. The high triglyceride synthetase activity of washed microsomes of pig and sheep tissue, however, indicates that much of the phosphatidate phosphohydrolase

content of the epithelial cell must be associated with this particulate fraction. As pointed out by Smith *et al.* (1967), the presence of the enzyme in the particle-free supernatant is probably the result of damage to the microsomes.

Most earlier workers (Brindley & Hübscher, 1965; Johnston *et al.* 1967) included GSH and potassium fluoride in their incubation media, but in our hands these components were without effect on the activities of tissue homogenates, and inhibited the triglyceride synthetase activity of microsomal fractions of pig and sheep tissue (Table 3). Inhibition of phosphatidate phosphohydrolase by fluoride was reported by Sedgwick & Hübscher (1967).

In the pig and the chicken, the microsomal fraction also synthesized triglycerides from fatty acid and monoglyceride, but in all subcellular preparations of sheep tissue, 1-monoglycerides were essentially ineffective as precursors of triglycerides. The failure of the pig homogenate to show triglyceride synthetase activity with monoglycerides was due to the presence in this fraction of an active

Number of carbon atoms and double bonds in fatty acid

Fig. 8. Effect of chain length and degree of unsaturation on the incorporation of fatty acids into triglycerides by the homogenates of sheep (a) and pig (b) intestinal epithelium.

lipase, detected by the release of radioactive oleic acid from glycerol mono[1-¹⁴C]oleate. This enzyme, a monoglyceride lipase, failed to hydrolyse glycerol tri[1-¹⁴C]oleate under identical conditions, and was found in the particle-free supernatant of all the species studied. Lipase activity has been demonstrated previously in pig (DiNella, Meng & Park, 1959), rat (Senior & Isselbacher, 1963) and chicken (Pope & Tidwell, 1964) intestinal mucosa. Dawson (1967) suggested that monoglyceride lipase may have a regulatory function in intestinal epithelial cells, excess of monoglyceride being diverted to the glycerol 3-phosphate of triglyceride synthesis. The presence of an active monoglyceride lipase in sheep intestinal epithelium may account for the monoglyceride radioactivity associated with the sheep total homogenate, although the possibility of direct incorporation of monoglyceride into triglyceride, as briefly reported by Leat & Cunningham (1968), cannot be excluded.

In the pig and chicken glycerol 1-mono-oleate was found to be the acyl fatty acid acceptor preferable to either glycerol 1-monopalmitate or glycerol 1-monostearate. Similar results were reported by Clark & Hübscher (1961) with the rabbit.

This investigation showed that the intestinal epithelia of the pig and chicken have a complete enzyme system for effecting triglyceride synthesis from free fatty acids and both glycerol 3-phosphate and monoglyceride, whereas in sheep tissue the glycerol 3-phosphate pathway predominates. These patterns of synthetic activity probably reflect the relative availability of monoglycerides to the mucosal cells of these species.

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