In Vivo Cholesterol and Fatty Acid Synthesis in the Pig Intestine[1] (35623)

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Recent experiments have demonstrated that a significant portion of the endogenous cholesterol pool may be synthesized in tissues other than the liver (1). While it is apparent that every mammalian tissue studied is capable of at least some degree of *de novo* cholesterol synthesis, the liver and intestine appear to have the highest rates of sterol synthesis in rats (2) and monkeys (3). There are few data on the relative rates of cholesterol synthesis in various human tissues. Preliminary results indicate that the rate of cholesterol synthesis by human intestinal slices approximates that of cholesterogenesis in human liver (1, 4). The importance of *de novo* cholesterol synthesis is emphasized by the fact that 60–75% of the serum cholesterol in men fed high-cholesterol diets may be from *de novo* synthesis (5).

O'Hea and Leveille (6) recently compared adipose tissue and liver as sites of nonsaponifiable lipid and fatty acid synthesis in the pig. They demonstrated that pig liver was essentially unable to convert glucose to nonsaponifiable lipid; however, if acetate was used as a substrate pig liver was capable of synthesizing nonsaponifiable lipids. Little synthesis of nonsaponifiable lipid occurred in pig adipose tissue (7) although this tissue appears to be the major organ for fatty acid synthesis in the pig (6). The relative importance of the intestine as a site for cholesterol and fatty acid synthesis in the pig is unknown. The studies to be reported were undertaken to evaluate the contribution of the intestine to cholesterol and fatty acid synthesis in the pig.

Methods. Six castrated male pigs of the Yorkshire breed were used. The pigs were fed a corn and soybean meal diet (8) and water *ad libitum.* Trace amounts of sodium acetate-1-^{14}C (60 μCi; 41 μCi/μmole) or sodium pyruvate-3-^{14}C (40 μCi; 2.1 μCi/μmole) were dissolved in 5 ml of physiological saline (0.9% NaCl) and injected into the vena cava. Twenty minutes after injecting the labeled substrate the pigs were killed by exsanguination (6). The liver was rapidly removed, weighed, and sampled. A 5-g sample of adipose tissue was obtained from the shoulder region. The intestine (pyloric–duodenal juncture to ileocecal juncture) was removed and the adventitia stripped off. After dividing the intestine into four equal lengths and flushing with water to remove the digesta, each segment was weighed. All tissues were placed on ice until frozen and were stored at —20° until analyzed.

Aliquot portions (150 mg) of liver and adipose tissue were weighed, placed in 10% KOH in methanol (w/v), and refluxed. The nonsaponifiable lipid and fatty acids were extracted (9). Cholesterol was isolated from the nonsaponifiable lipid as the digitonin-precipitable sterol (DPS) (10). In this paper DPS will be referred to simply as cholesterol; however, we are aware that small quantities of other sterols may also be present in DPS. Of the sterols in DPS, over 80% is cholesterol in rat gastrointestinal tract, liver, and adipose tissue (2). Radioactivity in liver and adipose tissue cholesterol and fatty acids was determined as previously described (9).

Each segment of the intestine was digested

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TABLE I. Body, Intestine, Liver, and Adipose Tissue Weights.

Substrate	Animal number	Body weight (kg)	Intestine weight (g)	Liver weight (g)	Adipose tissue weight[a] (g)
Acetate-1-^{14}C	1	36	1506	396	3600
	2	38	1253	365	3800
	3	31	1299	359	3100
	4	31	1227	394	3100
Pyruvate-3-^{14}C	5	87	1811	1362	8700
	6	85	1560	1242	8500

[a] Assumed to be 10% of body weight.

in warm HCl and the lipid was extracted (11). The intestinal lipid was then saponified and processed as described for the liver and adipose tissue.

The *in vivo* method used in these studies has obvious limitations. Leveille *et al.* (12) and Jansen *et al.* (13) have discussed some of the problems encountered in interpreting results from *in vivo* studies of this type. Transport of labeled intermediates and products from one compartment to another was minimized by killing the pigs 20 min after injecting the labeled substrate. O'Hea and Leveille (6) observed that counts in serum fatty acids were extremely low during a 40-min period after injecting a labeled substrate into pigs, suggesting that transport was minimal during this period.

Results. The body weight and the weight of the intestine, liver, and estimated adipose tissue mass is given in Table I for each of the six pigs used. The recovery of radioactivity from acetate-1-^{14}C in intestine, adipose tissue, and liver cholesterol and fatty acids is shown in Tables II and III, respectively. It is apparent from these results that liver and adipose tissue were responsible for a greater percentage contribution to overall lipogenesis than was the intestine. The relative contribution of various segments of the intestine to intestinal cholesterol and fatty acid synthesis is indicated in Table IV. The upper three-fourths of the intestine appears to be responsible for most of the incorporation of labeled acetate into cholesterol and fatty acids.

Pyruvate-3-^{14}C incorporation into fatty acids was primarily restricted to adipose tissue (91%) with the intestine and liver together contributing only 9% of the newly synthesized fatty acids (Table III). Pyruvate-3-^{14}C incorporation into cholesterol was too low to be quantitated in this experiment.

Discussion. The present study suggests that the liver is the major site of cholesterol synthesis in the fed pig consuming a low-cholesterol diet. The intestine, in these studies, contributed only about 4% to the newly synthesized cholesterol while the liver was responsible for 67%. This is in contrast to the recent observation (13) that *in vivo* incorporation of glucose-*U*-^{14}C into gastrointestinal tract cholesterol was four times greater than into liver cholesterol in mice fed a low-cholesterol diet. Further studies are needed to determine whether these differ-

TABLE II. *In Vivo* Incorporation of Acetate-1-^{14}C into Cholesterol by Pig Intestine, Liver, and Adipose Tissue.

Animal number	Total counts (10^3 dpm)			Contribution (%)		
	Intestine	Liver	Adipose tissue	Intestine	Liver	Adipose tissue
1	8	84	59	5	56	39
2	13	260	165	3	59	38
3	16	664	70	2	89	9
4	21	299	135	4	66	30
Mean				4	67	29

TABLE III. *In Vivo* Incorporation of Acetate-1-^{14}C or Pyruvate-3-^{14}C into Fatty Acids by Pig Intestine, Liver, and Adipose Tissue.

Substrate	Animal number	Total counts (10^3 dpm)			Contribution (%)		
		Intestine	Liver	Adipose tissue	Intestine	Liver	Adipose tissue
Acetate-1-^{14}C	1	50	1747	2988	1	37	62
	2	74	2180	2808	2	43	55
	3	43	2723	2790	1	49	50
	4	43	1254	2995	1	29	70
	Mean				1	40	59
Pyruvate-3-^{14}C	5	97	60	1035	8	5	87
	6	20	0	587	3	2	95
	Mean				6	3	91

ences can be attributed to the species studied or to the substrate utilized for cholesterol synthesis.

Each of the upper three segments of the pig intestine contributed more to intestinal cholesterol synthesis (mean = 29%) than did the lower segment (14%). This is in contrast to *in vitro* results obtained with rat and monkey intestine where sterol synthesis was two to three times greater in the ileum than in the jejunum (2, 3). Similarly (1) sterol synthesis is higher in ileum biopsy samples than in other portions of human intestine. In the present study each segment of the intestine weighed about the same but the ^{14}C-cholesterol content (dpm/g tissue) was lower in the proximal end. It remains to be established whether the results obtained in the pig vs. those obtained in the rat, monkey, and human can be attributed entirely to a species difference or whether the different techniques employed might have contributed to the differences observed.

TABLE IV. *In Vivo* Incorporation of Acetate-1-^{14}C into Cholesterol and Fatty Acids by Various Segments of the Pig Intestine.

Product	% Contribution: Segment of intestine[a]			
	1	2	3	4
Cholesterol	30 ± 2[b]	32 ± 2	24 ± 2	14 ± 1
Fatty acids	25 ± 3	33 ± 5	29 ± 3	13 ± 2

[a] Proximal to distal.

[b] Mean ± SEM of four pigs.

There is abundant evidence that the rate of cholesterol biosynthesis can be altered by the amount of cholesterol in the diet as well as by the caloric intake. High levels of cholesterol intake (rat, monkey, mouse, and man) and fasting (rat and monkey) result in a virtually complete suppression of hepatic cholesterol synthesis (1–3, 13). However, cholesterol synthesis in other tissues is suppressed to a much lesser degree by cholesterol feeding (2, 3, 13). Consequently in the cholesterol-fed animals extrahepatic cholesterol synthesis makes a very significant contribution to total-body cholesterol synthesis. The effect of feeding a high-cholesterol diet on the rates of cholesterogenesis in various tissues of the pig is unknown. Fasting results in increased serum cholesterol levels in the pig (14), and similar increases in serum cholesterol have been observed in humans fasted for several days (15). Further investigations into the role of high-cholesterol diets and fasting on the rates of cholesterogenesis in various tissues of the pig appear warranted.

Fatty acid synthesis by pig intestine represents only a small fraction (1–6%) of the estimated total synthesis. Fatty acid synthesis in intestinal rings and homogenates of rats (16) and guinea pigs (17) has also been demonstrated. The physiological significance of intestinal fatty acid synthesis has not been established. Tame and Dils (17) have speculated that it may be concerned with the structural needs of the cell or possibly play a role in supplying specific fatty acids re-

quired for ester synthesis. In the present study intestinal fatty acid synthesis rates were highest in the proximal three-fourths of the intestine corresponding to the area responsible for lipid absorption.

When acetate-1-^{14}C was used as a substrate, adipose tissue accounted for 59% and liver for 40% of the newly synthesized fatty acids. These results are in close agreement with those of O'Hea and Leveille (6), however, as they point out the quantitative significance of acetate to overall fatty acid synthesis in the pig has not been established.

When pyruvate-3-^{14}C was supplied as a substrate, adipose tissue accounted for about 90% of the newly synthesized fatty acids while the liver contributed only about 3%. This lends further support to the central role that adipose tissue plays in fatty acid synthesis in the pig. When glucose-*U*-^{14}C was a substrate for fatty acid synthesis in the pig, adipose tissue accounted for more than 99% of the newly synthesized fatty acids (6). Since glucose is likely the predominant substrate absorbed, results obtained with pyruvate-3-^{14}C and glucose-*U*-^{14}C probably more nearly reflect the actual situation than results obtained when acetate-1-^{14}C was used as a substrate for fatty acid synthesis in the pig.

When glucose or pyruvate are substrates for fatty acid synthesis, citrate cleavage enzyme is necessary for the production of acetyl CoA. As discussed by O'Hea and Leveille (6), the very low activity of citrate cleavage enzyme in pig liver is undoubtedly an important factor contributing to the liver's low capacity for fatty acid synthesis from glucose as well as from pyruvate.

Summary. The results of these studies show that in the pig the intestine contributes only about 4% to cholesterol synthesis from acetate-1-^{14}C while liver and adipose tissue contribute 67 and 29%, respectively. Each of the proximal three segments of the intestine contributed more to cholesterol synthesis than did the distal segment. Fatty acids were synthesized primarily in the adipose tissue of the pig when pyruvate-3-^{14}C was the substrate while the intestine and liver together contributed only about 9% to overall fatty acid synthesis. Fatty acid synthesis from acetate-1-^{14}C in pig intestine was of minor importance when compared with liver or adipose tissue fatty acid synthesis.

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