The Form of Absorption of Lipids in the Chicken, *Gallus domesticus*[1] (36878)

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It has been conclusively demonstrated that long chain fatty acids in mammals are absorbed mainly in the lymphatic system in the form of chylomicrons (1, 2). Chylomicrons are defined operationally as those particles with S_f values larger than 400 containing triglyceride primarily of dietary origin (1). In birds the route and form of absorption of lipids has not been as clearly defined.

The lymphatic system in birds is not as well developed as that of mammals. Long (3) found no evidence of milky lymph in birds after eating. Noyan *et al.* (4), following injection of palmitic acid-1-^{14}C into the gizzard, found ratios of lipid-^{14}C in portal vein blood to systemic blood greater than one, demonstrating that palmitic acid is absorbed via the portal route. These authors also separated two pancreaticoduodenal plasma samples into two density classes: $S_f > 20$ and $S_f < 20$. They reported that 88% of palmitic-1-^{14}C was present in the $S_f > 20$ and mainly in the triglyceride form. The previous experimental approach suffers from several limitations. Prior to blood sampling the birds were anesthetized. Anesthesia has been shown to affect absorption, secretion and enzymatic activity (5). Another major limitation in interpreting the centrifugal and composition data is that no correction was made for recirculation of labeled palmitic-1-^{14}C. Therefore some of the absorbed palmitic acid could have been absorbed as fatty acid, taken up by parenchymal liver cells, and secreted as $S_f > 20$ particles in the form of triglyceride. Peripheral hydrolysis of the absorbed particles was not considered either.

We have reexamined this problem of the form of absorption of lipids in birds by studying absorption in the conscious functionally hepatectomized rooster in the presence and absence of a lipoprotein lipase inhibitor Triton WR 1339.

Material and Methods. Eight to 10-mo-old white leghorn roosters were used in these studies. One week to 10 days before the experimental day, loose ligatures were placed around the afferent vessels to the liver (the right and left portal veins and associated afferent arteries) and brought to the skin surface. A functional hepatectomy can thus be produced instantly, and whenever desired, by tightening the exteriorized ligatures in a bird free from the effect of anesthesia and surgical trauma (6).

Ligations of the portal vein in birds does not affect the blood supply to the small intestine and presumably absorption, because of the existence of a blood vessel, peculiar to birds, the coccygeomesenteric vein (7) which links the portal vein to the renal portal system (Fig. 1). In effect, this vessel functions as a portal vein–posterior vena cava shunt. In such conscious, functionally hepatectomized roosters, micellar solutions (8) of oleic acid-1-^{14}C were infused in the duodenum via an indwelling polyethylene catheter (PE 80). In some experiments, Triton WR 1339 (Ruger Chemical Corp.), a known inhibitor (9) of lipoprotein lipase, was injected intravenously prior to infusion.

The infusion solution was prepared by sonicating at maximum energy (Branson sonifier) for 2 min at 20°: 3 ml of distilled water, 3 ml of 0.15 *M* phosphate buffer adjusted to pH 6.3, 6 ml of 0.04 *M* Na taurocholate in 0.15 *M* NaCl, 19.2 mg of glycerol-1-oleate and 45.7 mg of oleic acid. In addition, 50 μCi

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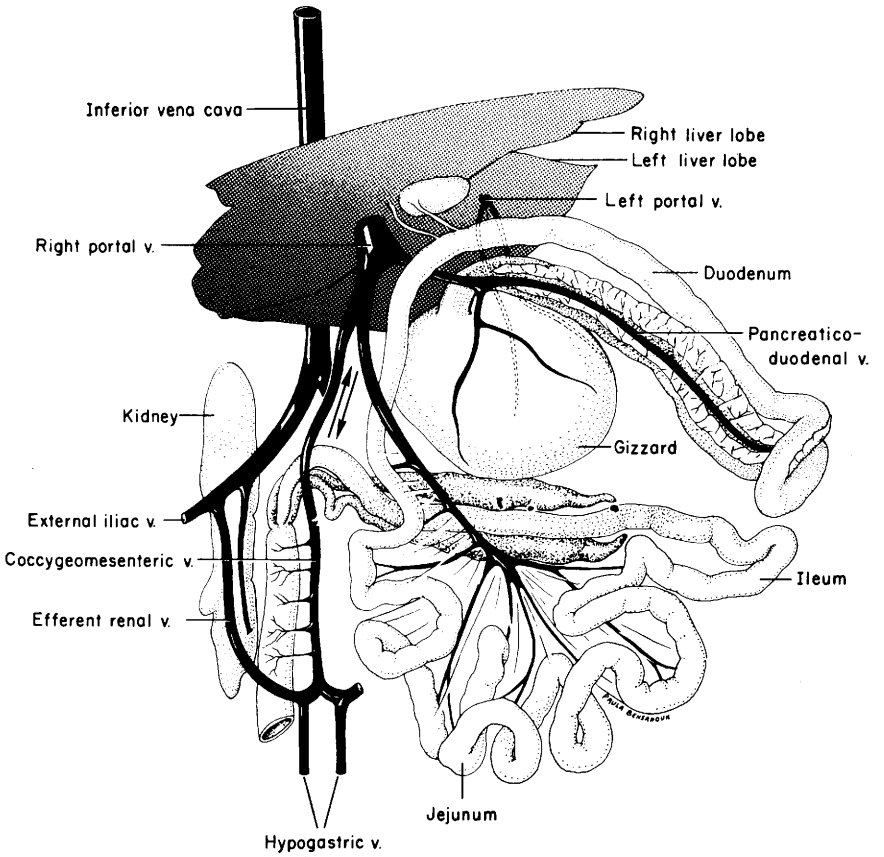

FIG. 1. Afferent vessels to the chicken liver. This diagram was obtained following latex injection of the abdominal vessels. The coccygeomesenteric vein links in birds the kidney and liver portal systems. When ligatures are placed around the right and left portal veins, blood draining the visceral organs flow through the coccygeomesenteric vein to the efferent renal vein and the inferior vena cava.

of oleic acid-1-^{14}C (New England Nuclear Corp.) was included in this mixture before sonication.

The micellar solution was infused at a rate of 0.2 ml/min and infusion continued for approximately 1 hr at which time a large blood sample for various analyses was collected by heart puncture. To obtain a gross estimate of lipid absorption the small intestine was dissected and flushed with 50 ml of saline and the intestinal contents and washed intestine saved for radioactivity measurements. Lipoprotein classes were separated by ultracentrifugation (10). Lipid extraction and purification were conducted by the method of Folch, Lees and Sloane Stanley (11). Lipid extraction of gut contents was conducted on frozen dried material and the Folch wash was omitted since loss of fatty acid radioactivity in the upper phase was experienced in the presence of bile salts. Lipoprotein fractions in high salt concentrations were extracted with 50 to 100 vol of solvent mixture. Lipid classes were separated by thin layer chromatography on silica gel H with a double-development procedure which allowed separation of diglycerides from triglycerides, and monoglycerides from phospholipids (12). The separated lipid bands were scraped, transferred to counting vials and counted by the method of Gordon and Wolfe (13).

Results. The technique of ligation of afferent vessels to the liver was successful in pro-

TABLE I. Fate of Oleic Acid-1-^{14}C Infused as a Micellar Solution in the Duodenum of Functionally Hepatectomized Chickens.

Obs	Intestinal contents %[a]	Gut wall %	Liver %
6 (hepat.)	22.7 ± 5.8	11.5 ± 5.3	1.0 ± 0.4
5 (hepat. + Triton)	24.0 ± 8.0	13.4 ± 4.4	1.9 ± 0.5

[a] Percentage of dose.

ducing an effective hepatectomy since less than 2% (Table I) of the infused dose was present in the liver after infusion. When labeled oleic acid was infused into nonhepatectomized birds, nearly 50% of the infused radioactivity was recovered in the liver after 30 min. In the conscious functionally hepatectomized rooster absorption occurred normally since less than 25% of the infused dose was recovered in the gut lumen.

In hepatectomized birds, in the presence of Triton WR 1339 most of the oleic-1-^{14}C radioactivity was present in the triglyceride fraction, whereas in its absence a significant amount of oleic-1-^{14}C radioactivity was present in the free fatty acid class (Table II). The oleic-1-^{14}C radioactivity present as free fatty acids ranged from 5 to 22%. When the detergent was present, close to 90% of the plasma radioactivity was associated with the very low density lipoprotein (VLDL) class ($d < 1.006$) (Table III). When the detergent was omitted only 36% of the radioactivity appeared in the VLDL fraction, and significant percentages of radioactivity were present in the low and high density lipoproteins.

Discussion. Functional hepatectomy and the use of an inhibitor of lipoprotein lipase, Triton WR 1339 provides a satisfactory technique to collect from the plasma the lipoproteins secreted during lipid absorption. Intestinal absorption is maintained in the hepatectomized bird because of the drainage of intestinal venous blood via the coccygeomesenteric vein (Fig. 1). Hepatectomy prevents the uptake of labeled free fatty acids and their secretion as very low density lipoproteins.

When lipoprotein lipase was inhibited in the presence of detergent, most of the oleic acid-1-^{14}C appeared in the peripheral blood as triglyceride. Infused oleic acid was therefore esterified in the gut wall and secreted as triglyceride in the form of a lipoprotein floating at a density less than 1.006. Preliminary analyses indicate that these lipoproteins have a chemical composition similar to mammalian chylomicrons. The following percentage composition was obtained for a large pooled sample: Phospholipid, 1.0; cholesterol 3.8; cholesterol ester, 1.8; monoglyceride, 0.2; diglyceride, 1.4; triglyceride, 91.0; and protein, 0.8%. Particle size determinations by sucrose density gradient (14) gave a distribution with a mode of 150 nm. It is suggested that these lipoproteins rich in exogenous fatty acids be named portomicrons since they are absorbed via the portal system. This term is selected for avian species by analogy to the term chosen by Gage and Fish (15) in mammals for particles rich in dietary lipids, chylomicrons, which are ab-

TABLE II. Distribution of Labeled Oleic Acid-1-^{14}C in Various Plasma Lipid Classes in Functionally Hepatectomized Roosters.

Obs	PL[a] %	M %	DG %	FA %	TG %	CE %
5 (hepat.)	2.9 ± 0.9	1.6 ± 0.3	2.3 ± 0.9	12.8 ± 3.0	79.8 ± 3.5	0.8 ± 0.3
5 (hepat. + Triton)	1.4 ± 0.8	1.7 ± 1.0	3.9 ± 2.7	3.7 ± 0.7[b]	88.9 ± 2.3	2.2 ± 0.9

[a] PL = phospholipid, M = monoglyceride, DG = diglyceride, FA = fatty acid, TG = triglyceride, CE = cholesterol ester.

[b] $p < .05$.

TABLE III. Distribution of Labeled Oleic Acid-1-^{14}C in Various Lipoprotein Classes in Functionally Hepatectomized Chickens.

	Density			
Obs	<1.006 %	1.006–1.063 %	1.063–1.21 %	>1.21 %
6 (hepat.)	36.1 ± 6.9	4.9 ± 0.7	27.0 ± 7.8	29.9 ± 5.0
5 (hepat. + Triton)	88.0 ± 3.8[b]	6.1 ± 1.5	2.8 ± 0.7[a]	4.4 ± 1.3[b]

[a] $p < .05$.
[b] $p < .01$.

sorbed into the chyle via lymphatic capillaries.

In the absence of detergent, when the absorbed exogenous triglycerides were partially metabolized, a significant proportion of radioactivity appeared in the free fatty acid fraction (Table II). This suggests that as the lipoprotein lipase of extrahepatic tissues interacts with the exogenous triglyceride-rich lipoproteins, fatty acids released are not all absorbed into adipocytes or other peripheral tissues. A significant amount of free fatty acids remains within the capillaries and continues on its way to appear in the venous outflow. The functional hepatectomy prevents the normal reesterification of free fatty acids in the liver, and results in their accumulation in the plasma. The plasma VLDL pool might represent a significant precursor pool of plasma free fatty acids.

In the absence of detergent, the surgical preparation previously described, allows the peripheral degradation of portomicrons and very low density lipoproteins. The degraded particles accumulate in the plasma where they can be sampled at various time intervals. This preparation is more physiological than the hepatectomized rat (16) since in the former blood flow through the abdominal viscera is maintained.

Summary. It has been demonstrated with the use of a functionally hepatectomized preparation and in the presence of a lipoprotein lipase inhibitor, Triton WR 1339, that, in the chicken, long chain fatty acids are absorbed in the form of triglycerides as the major component of a lipoprotein of density less than 1.006.

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