Pathway and form of absorption of palmitic acid in the chicken

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SUMMARY The ratio of lipid C^{14} in plasma of portal vein blood to that in plasma of systemic blood was determined in chickens killed 30, 60, 90, and 150 min after injection of palmitic acid-l-C^{14} into their gizzards. In other experiments other ratios were determined: (*a*) lipid C^{14} in plasma of pancreaticoduodenal blood to that in portal vein blood in chickens killed 30 min after fatty acid administration; and (*b*) lipid C^{14} in jejunal vein to that in portal vein plasma in birds killed 90 and 150 min after the injection. The results demonstrated that the portal system is a significant pathway for the absorption of fatty acid from the intestines of the bird, and that during the early period absorption from the duodenum takes place.

Measurement of the C^{14} remaining in the digestive tract contents indicated that up to 96% of the injected labeled palmitic acid was absorbed in 90 min. Analysis of ultracentrifugally-separated plasma lipoproteins in blood drawn from the pancreaticoduodenal vein half an hour after introduction of the labeled palmitic acid into the gizzard revealed that the palmitic acid was absorbed from the intestines into the bloodstream principally as triglycerides in the very low density ($S_f > 20$) lipoproteins.

In MAMMALS, long-chain fatty acids are absorbed almost exclusively by way of the lymph (1–4). Although the bird's lymphatic system is not nearly as well developed as is the mammal's, Hewson (5), who was the first to describe in some detail the lymphatic system in the bird, referred to its intestinal lymphatics as lacteals. Kaupp (6) and Bolton (7) have stated that the intestinal lymphatics of the bird take up emulsified fat. Several years ago, in this laboratory, Kiyasu (8) examined microscopic sections of the jejunal villi of chickens after perfusing the small intestines with India ink, and noted that their structure was unlike that of the mammalian villus. The capillary network, rather than being peripherally located with ample room in the villus core for a central lacteal, occupied virtually the entire core—a structural design thought by Kiyasu to favor the absorption of all nutrients via the portal system. Because of their minute calibre, he could not cannulate the chicken's lymphatics. Kiyasu therefore measured the ratio portal plasma lipid C^{14}: systemic plasma lipid C^{14} in birds fed C^{14}-labeled fatty acids. His finding of values greater than unity led him to suggest that in the bird fatty acids are absorbed via the portal system.

Further studies on the absorption of fatty acid from the intestines into the circulation in the chicken are reported here. The most convincing evidence for the absorption of fatty acid via the portal system was obtained in experiments in which pancreaticoduodenal vein : portal vein and portal : systemic lipid C^{14} ratios were determined after injection of palmitic acid-l-C^{14} into the gizzards. In order to determine the form in which the labeled fatty acid was absorbed into the bloodstream we determined the distribution of C^{14} among lipid fractions of the lipoprotein classes $S_f > 20$ and $S_f < 20$, which were isolated ultracentrifugally from the plasma of blood drawn from the pancreaticoduodenal vein at a time when absorption of the administered labeled palmitic acid from the duodenum into that vein was taking place.

EXPERIMENTAL METHODS AND MATERIALS

Before embarking on the experiments described below attempts were made to cannulate the lymphatic vessels of the chicken. These attempts were unsuccessful.

Palmitic acid-l-C^{14} with a specific activity of 8.5 mc/mmole was purchased from Calbiochem Inc., Los Angeles, Calif., and purified on a silicic acid column (9).

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Administration of Palmitic Acid and Blood Sampling

White Leghorn hens and cockerels, weighing 1100–2000 g, that had been maintained on Purina Broiler Chow were used. An oblique one-half inch incision was made in the left lateral abdominal wall at a point overlying the gizzard. Twenty microcuries of the palmitic acid-1-C^{14}, dissolved in 0.8 ml of corn oil, were injected into the exposed gizzard, and the incision was sutured. The birds were restrained by hand and were not unduly excited while the above procedure was carried out; the technique is in accordance with the common poultry-farming practice of caponization via an abdominal incision, and does not seem to be harmful to the animal. Ten minutes before blood samples were taken, the birds were anesthetized by slow, intravenous injections of 0.3–0.6 ml of a 2% solution of sodium pentobarbital (Abbott Laboratories, Chicago, Ill.). The injections were discontinued when the animals no longer reacted to pinching of the comb. The abdominal wall was then opened on the right side, exposing the intestines. The coccigeomesenteric vein, a systemic vein that joins the portal vein near the liver, was clamped, and 10 ml of blood were drawn into a heparin-rinsed syringe from the portal vein at point *a* shown in Fig. 1. This point was found to be the closest to the liver at which venipuncture could be done without rupturing the vessel and without extensive dissection. Another 10 ml of blood were immediately drawn from the heart, following which the chickens were killed. In this way samples of portal and systemic blood were taken at either 30, 60, 90, or 150 min after administration of the labeled palmitic acid. In some experiments, blood samples were also drawn from the pancreaticoduodenal vein (point *c* in Fig. 1) at 30 min or from the jejunal vein (point *b*) at 90 and 150 min.

Treatment of Blood Samples and Ultracentrifugal Separation of Plasma Lipoproteins

The blood was centrifuged, and aliquots of plasma were placed in ethanol–diethyl ether 3:1 (v/v). Three milliliters of certain of the pancreaticoduodenal vein plasma samples were transferred to Spinco ultracentrifuge tubes. The volume was adjusted to 9 ml with a NaCl solution of density 1.006 g/ml, and the mixtures were centrifuged at 79,400 × *g* for 20 hr at 4°. The top 2 ml containing the lipoprotein classes of $S_f > 20$, the bottom 5 ml containing those of $S_f < 20$, and the 2 ml separating these two lipoprotein fractions were transferred to 3:1 ethanol–ether (v/v). (The middle layer was taken to determine the completeness of the ultracentrifugal separations.)

Extraction and Chromatographic Analysis of Plasma Lipids

Lipids were extracted from plasma and plasma fractions as described in (10). The C^{14} lipids of the $S_f >$ 20 and $S_f < 20$ lipoproteins were separated on silicic fractions: (*a*) cholesterol esters; (*b*) one that contained triglycerides, mono- and diglycerides, and free fatty acids; and (*c*) phospholipids. Florisil columns (11) were used to separate fraction (*b*) into triglycerides, mono- plus diglycerides, and free fatty acids.

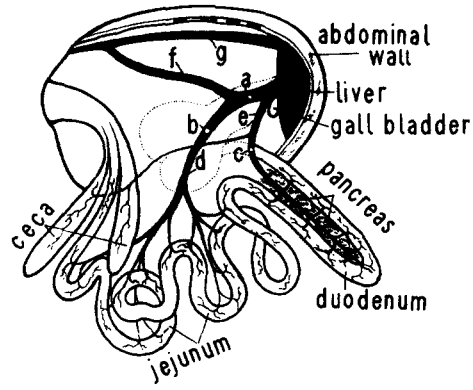

FIG. 1 Tributaries of the portal vein in the chicken and points of venipuncture for blood sampling: *a*, point of venipuncture for portal sampling; *b*, point of venipuncture for jejunal sampling; *c*, point of venipuncture for pancreaticoduodenal sampling; *d*, jejunal vein; *e*, pancreaticoduodenal vein; *f*, coccigeomesenteric vein; and *g*, caudal vena cava.

Procedure for Determining Percentage of Palmitic Acid-1-C^{14} Absorbed

The percentage of the isotopic palmitic acid absorbed was determined in two chickens from which portal and systemic blood samples were drawn 90 min after injection of the labeled palmitic acid into their gizzards, and in a third from which blood samples were obtained 150 min after the injection. After removal of the blood samples the birds were killed, and their digestive tracts, from the gizzard to the cloaca, were excised. The contents were washed out of the digestive tracts with 1–2 liters of warm tap water, and lyophilized. The lipids were extracted from the dried residue with ethanol–ether 3:1 in the same manner as from plasma. The C^{14} absorbed was calculated by subtracting the C^{14} recovered in the digestive tract from the C^{14} that was injected. Immediately after injection of the labeled palmitic acid, 100% of the C^{14} was recovered by this procedure.

C^{14} Assay

Dried lipid samples were dissolved in 15 ml of a toluene solution containing 45 mg of 2,5-diphenyloxazole and 1.5 mg of 1,4-bis-2-(5-phenyloxazolyl)benzene, and assayed for C^{14} in a Packard Tri-Carb liquid scintillation spectrometer.

RESULTS AND DISCUSSION

The values for the portal:systemic plasma lipid C^{14} ratio were greater than 1.0 (Table 1) for all birds from

TABLE 1 PORTAL:SYSTEMIC PLASMA LIPID C^{14} RATIOS AT VARIOUS INTERVALS AFTER INJECTION OF PALMITIC ACID-1-C^{14} INTO CHICKEN GIZZARDS

Min after Injection of Palmitic Acid	Portal:Systemic Ratio*	
	Range	Average
30	1.3–1.6 (4)†	1.5
30	0.3–0.9 (5)	0.6
60	2.3–2.8 (2)	2.6
90	1.1–1.8 (10)	1.4
150	1.0–2.1 (6)	1.4

* $\frac{\text{Total lipid } C^{14} \text{ per ml plasma in portal vein blood}}{\text{Total lipid } C^{14} \text{ per ml plasma in systemic blood}}$

† The figures in parentheses are the number of birds from which blood samples were drawn.

which blood samples were taken at 60 and 90 min, and for 5 of the 6 birds at 150 min after the injection of the labeled palmitic acid into the gizzard. They were <1.0 for 5 of the 9 birds from which blood samples were taken 30 min after introduction of the palmitic acid into their gizzards.

To account for values of <1.0 in the 5 birds at 30 min, the possibility was considered that, at that time, these chickens were absorbing the labeled fatty acid into the bloodstream almost exclusively from the duodenum. Figure 1 shows that, if a bird absorbs the labeled palmitic acid for the most part from the duodenum, the C^{14} content of blood at *a* (the point at which the portal blood samples were drawn) would be lower than that at *c* in the pancreaticoduodenal vein. Under such circumstances, the C^{14} content of the portal blood at point *a* would very likely also be lower than that of the portal blood between the liver and the junction of the portal and the pancreaticoduodenal veins, and might also be lower than that of the blood leaving the liver. If, on the other hand, the absorption of the labeled fatty acid is no longer taking place chiefly from the duodenum, and is taking place below it, the C^{14} content of the pancreaticoduodenal vein and of the portal vein between the liver and the junction of the portal and pancreaticoduodenal veins could be lower than that of the portal blood taken at *a*. If the assumptions made above are valid, a value for the portal:systemic C^{14} ratio of <1.0 would be associated with a value for the pancreaticoduodeanl vein:portal vein ratio of >1.0; and a portal:systemic ratio of >1.0 could be associated with a pancreaticoduodenal:portal ratio of <1.0 when blood samples are taken 30 min after injection of the labeled palmitic acid into the gizzard.

TABLE 2 PANCREATICODUODENAL VEIN:PORTAL VEIN PLASMA LIPID C^{14} RATIOS AND PORTAL:SYSTEMIC PLASMA LIPID C^{14} RATIOS AT 30 MIN AFTER INJECTION OF C^{14}-LABELED PALMITIC ACID INTO CHICKEN GIZZARDS

Pancreaticoduodenal: Portal Ratio	Portal:Systemic Ratio
13.30	0.60
5.25	0.30
0.63	1.30

TABLE 3 JEJUNAL VEIN:PORTAL VEIN PLASMA LIPID C^{14} RATIOS AND PORTAL:SYSTEMIC PLASMA LIPID C^{14} RATIOS AT 90 AND 150 MIN AFTER INJECTION OF PALMITIC ACID-C^{14} INTO CHICKEN GIZZARDS

Min After Injection	Jejunal:Portal Ratio	Portal:Systemic Ratio
90	2.05	1.20
90	2.34	1.60
150	2.17	1.50

Table 2 shows the values for pancreaticoduodenal:portal and for portal:systemic plasma lipid C^{14} ratios for 3 chickens from which blood samples were taken 30 min after administration of the labeled palmitic acid. In 2 of the 3 birds values for the portal:systemic ratio <1.0 were observed, and in both of them the values for the pancreaticoduodenal vein:portal vein C^{14} ratio greatly exceeded 1. In the third bird the value for the portal:systemic ratio was >1.0 and that for the pancreaticoduodenal:portal ratio was <1.0. Apparently, then, in two of the birds absorption was taking place chiefly from the duodenum, while in the third it was taking place, for the most part, below the duodenum. At 90 and 150 min after injection of the labeled palmitic acid into the gizzard, at which times values for the portal:systemic C^{14} ratio >1.0 were consistently observed, it would be expected that values for jejunal vein (*d*, Fig. 1):portal vein C^{14} ratio >1.0 would be observed. This proved to be the case with three birds—two from which blood samples were drawn at 90 min and one from which samples were obtained at 150 min (Table 3).

The concentration of lipid C^{14} in the pancreaticoduodenal and jejunal veins in the experiments described above was about the same. This observation suggests that an appreciable amount of the injected palmitic acid was absorbed into the bloodstream from the duodenum. This might seem surprising—provided bile and pancreatic juice are important for fat absorption in the bird—because both bile and pancreatic ducts open into the caudal end of the duodenum. There is evidence, however, for the occurrence of antiperistalsis

TABLE 4 PERCENTAGES OF PALMITIC ACID-C^{14} ABSORBED FROM THE GASTROINTESTINAL TRACT 90 AND 150 MIN AFTER INJECTION INTO CHICKEN GIZZARDS

Min after Injection	% of Palmitic Acid-C^{14} Absorbed
90	96
90	88
150	89

TABLE 5 DISTRIBUTION OF LIPID C^{14} IN PANCREATICODUODENAL VEIN 30 MIN AFTER INJECTION OF PALMITIC ACID-1-C^{14} INTO CHICKEN GIZZARDS

% of Plasma Lipid C^{14} Recovered as:		% of Lipoprotein Lipid C^{14} Recovered in Chromatographically Separated Fractions							
		$S_f > 20$ Lipoproteins				$S_f < 20$ Lipoproteins			
$S_f > 20$ Lipoproteins	$S_f < 20$ Lipoproteins	Cholesterol Esters	Glycerides*	Free Fatty Acids	Phospholipids	Cholesterol Esters	Glycerides*	Free Fatty Acids	Phospholipids
89.9	10.1	1.9	91.8	5.1	1.2	6.3	70.4	15.8	7.5
86.5	13.5	1.3	94.6	3.1	1.0	3.8	78.5	8.9	8.8

* Less than 1% of the glyceride C^{14} was recovered in the mono- and diglyceride fractions.

in the duodenum, which would result in mixing bile and pancreatic juice with the duodenal contents (12). Furthermore, since the duodenum of the pigeon, according to Verzar and McDougall (13), contains one third of the total intestinal surface, one might expect it to be an important site of fat absorption in birds.

The percentages of the labeled palmitic acid absorbed from the intestines—determined for two of the birds from which blood samples were taken 90 min after injection of the labeled fatty acid into their gizzards and from another from which samples were taken at 150 min—are shown in Table 4. As much as 96% of the administered, C^{14}-labeled palmitic acid was absorbed by this time. Similar values for the absorption of C^{14}-labeled palmitic acid in the bird were observed by Kiyasu (8).

Analysis of plasma lipoproteins separated ultracentrifugally from blood drawn from the pancreaticoduodenal veins of birds that were evidently absorbing the C^{14}-labeled palmitic acid from the duodenum 30 min after its administration (the two with high pancreaticoduodenal:portal ratios) indicated that almost 90% of the fatty acid-C^{14} absorbed was in the very low density ($S_f > 20$) lipoproteins (Table 5). Over 90% of the lipid C^{14} in these very low density lipoproteins was in the form of triglycerides (Table 5). A maximum of 5% was recovered as free fatty acids, and from 1 to 2% in each of the other fractions—cholesterol esters and phospholipids. The higher density lipoproteins ($S_f < 20$) contained a somewhat greater proportion of the lipid C^{14}—up to 30%—as free fatty acids, cholesterol esters, and phospholipids. Thus the principal form in which the fatty acid was absorbed into the circulation was as triglycerides of very low density lipoproteins.

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