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In Vivo Lipogenesis in the Domestic Chicken (33022)

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The metabolism of mammalian adipose tissue has received considerable attention in recent years. Numerous studies have demonstrated that rat adipose tissue can synthesize large quantities of fatty acids *in vitro* (1). In fact, adipose tissue appears to be the major site of fatty acid synthesis in the mouse (2) and rat (3,4). In contrast to studies with mammalian species, avian adipose tissue has received little attention. Goodridge and Ball reported that pigeon adipose tissue had a very low lipogenic capacity (5) and similar results have been obtained with chicken adipose tissue (6). *In vivo* studies have led to the conclusion that pigeon adipose tissue accounts for only about 4% of total fatty acid synthesis, while liver is responsible for the remainder (7). In view of the low *in vivo* lipogenic capacity of chicken adipose tissue (6), it might be anticipated that in this species, as reported for the pigeon, liver is the major site of fatty acid synthesis. The studies reported were undertaken to evaluate the relative importance of liver and adipose tissue as sites of fatty acid synthesis in the chicken.

Methods. Male crossbred chicks (New Hampshire ♂ × Columbian ♀) weighiing 490–630 gm were used. The chicks were fed a semipurified diet similar in composition to that described earlier (8). On the day of experiment the chicks were given glucose-U-^{14}C or acetate-1-^{14}C intraperitoneally in 1 ml of saline. Thirty and 60 min after administration of the tracer, blood was obtained by cardiac puncture with a heparinized syringe. The animals were then killed by cervical dislocation and the liver and adipose tissues were rapidly removed and cooled on ice. Adipose tissue was obtained from the neck region. Lipid was extracted with chloroform: methanol (2:1, v/v) as previously described (9). A portion of the lipid extract was taken for liquid scintillation counting. The remainder of the lipid extract was evaporated to dryness and saponified by refluxing with 10 ml of methanolic KOH (5%). Following saponification the solution was diluted with 10 ml H_2O and the nonsaponifiable lipids were removed with 3 successive 5-ml portions of petroleum ether (bp 30–60°). The fatty acids were removed in a similar manner after acidification of the aqueous fraction with concentrated HCl. The samples were prepared for liquid scintillation counting by evaporating to dryness and dissolving the lipids (total lipids, nonsaponifiable lipids, or fatty acids) in 10 ml of a toluene scintillant (6).

Results. The results of two experiments designed to study the ability of the chick to utilize glucose for lipid synthesis are presented in Table I. The results of these two experiments are in agreement and show that the chick can readily incorporate glucose carbon into lipids. The amount of radioactivity in fatty acids was higher in liver than in adipose tissue at both time periods studied. However, during the first 30 min the incorporation of glucose into total lipids was greater in adipose tissue than in liver but only 2–4% of this radioactivity could be accounted for in fatty acids and nonsaponifiable

TABLE I. *In Vivo* Incorporation of Glucose-U-^{14}C into Chick Liver, Adipose Tissue, and Plasma Lipids.[a]

Measurement	Time elapsed between glucose-U-^{14}C administration and killing of chicks (min)			
	Expt. I[b]		Expt. II[b]	
	30	60	30	60
Body wt. (gm)	510 ± 15	490 ± 13	540 ± 27	540 ± 11
Liver lipid-^{14}C (10^3 dpm/gm of liver)				
Total lipid	18.94 ± 1.27	38.91 ± 3.32	23.35 ± 8.89	43.36 ± 9.34
Fatty acid	3.85 ± 2.22	25.40 ± 4.24	9.56 ± 8.02	22.94 ±11.95
Nonsaponifiable lipid	1.40 ± 0.89	7.47 ± 1.10	0.98 ± 0.78	1.51 ± 0.69
Unaccounted for (%)	72	15	55	27
Adipose tissue lipid-^{14}C (10^3 dpm/gm of tissue)				
Total lipid	52.18 ±18.75	6.46 ± 2.61	31.44 ±18.10	9.39 ± 2.38
Fatty acid	0.78 ± 0.11	3.88 ± 2.12	1.23 ± 0.52	4.45 ± 2.65
Nonsaponifiable lipid	0.04 ± 0.02	0.10 ± 0.02	0.06 ± 0.02	0.07 ± 0.02
Unaccounted for (%)	98	38	96	52
Plasma lipid-^{14}C (10^3 dpm/ml)				
Total lipid	6.17 ± 2.91	1.84 ± 0.37	3.98 ± 0.67	3.17 ± 0.67
Fatty acid	0.05 ± 0.02	0.40 ± 0.25	0.35 ± 0.32	0.46 ± 0.24
Nonsaponifiable lipid	0.02 ± 0.01	0.19 ± 0.04	0.03 ± 0.03	0.06 ± 0.03
Unaccounted for (%)	99	68	90	84

[a] Chicks received glucose-U-^{14}C intraperitoneally in 1 ml of saline: each chick received 7.4 μC in Expt. I and 7.9 μC in Expt. II.

[b] Values are means ± SEM for 4 chicks in Expt. I and 5 chicks in Expt. II.

lipids of adipose tissue. The radioactivity which could not be accounted for was presumably in the glycerol moiety of the lipids. In fact, glycerol was isolated from some samples and the radioactivity could be accounted for in this fraction. In plasma, 68–99% of the total lipid radioactivity was in this fraction, unaccounted for by fatty acid and nonsaponifiable lipids.

The two time periods used in the experiments presented in Table I enable an estimation to be made of rates of fatty acid synthesis. Such calculations show that the rate of increase of radioactivity in fatty acids was greater in liver than in adipose tissue. Significant radioactivity in the nonsaponifiable lipid fraction was observed only in liver. In both experiments the radioactivity in the fatty acid and nonsaponifiable lipid fraction of plasma increased between 30 and 60 min after glucose-U-^{14}C administration.

Since *in vitro* studies had shown acetate to be a better substrate for fatty acid synthesis than glucose (6), the utilization of acetate-1-^{14}C was also studied as shown in Table II. The results of this experiment are in general agreement with those presented in Table I. Per unit tissue weight, liver lipids contained considerably more radioactivity than adipose tissue lipids. Also, the rate of increase of fatty acid radioactivity between 30 and 60 min after acetate-1-^{14}C administration was greater in liver than in adipose tissue by a factor of about 5. The radioactivity in plasma lipids increased between 30 and 60 min after acetate administration in a manner similar to that observed when glucose-U-^{14}C was used (Table I).

Discussion. The relative importance of adipose tissue and liver as sites of fatty acid synthesis in the chick can be estimated from the data presented. In all experiments, total liver weight was determined to be 2.3–2.5% of body weight. In Expt. I (Table I) total body fat was determined and found to be 6.8% of body weight. Using these values the total fatty acid radioactivity in liver and adipose tissue was calculated for the two

TABLE II. *In Vivo* Incorporation of Acetate-1-^{14}C into Chick Liver, Adipose Tissue, and Plasma Lipids.[a]

Measurement	Time elapsed between acetate-1-^{14}C administration and killing of chicks (min)	
	30	60
Body wt. (gm)	630 ± 19[b]	635 ± 18
Liver lipid-^{14}C (10^3 dpm/gm of tissue)		
Fatty acid	16.14 ± 2.94	27.63 ± 6.76
Nonsaponifiable lipid	7.74 ± 2.27	7.56 ± 1.65
Adipose tissue-^{14}C (10^3 dpm/gm of tissue)		
Fatty acid	1.80 ± 0.36	4.14 ± 1.14
Nonsaponifiable lipid	0.05 ± 0.02	0.05 ± 0.01
Plasma lipid-^{14}C (10^3 dpm/ml)		
Fatty acid	0.70 ± 0.11	1.31 ± 0.32
Nonsaponifiable lipid	0.26 ± 0.07	0.53 ± 0.15

[a] Each chick received 4.08 μC of acetate-1-^{14}C intraperitoneally.
[b] Mean ± SEM for 5 chicks.

periods studied. These calculations show that liver accounts for 64–75% of the total fatty acids synthesized or, conversely, that adipose tissue accounted for 25–36% of fatty acid synthesis. The results obtained for the different experiments agreed quite well. Experiments I and II (Table I) in which glucose-U-^{14}C was used, yielded average values of 30 and 31%, respectively, as the contribution of adipose tissue to total fatty acid synthesis. When acetate-1-^{14}C was used (Table II) adipose tissue accounted for 33% of the total fatty acids synthesized. Thus, these data indicate that, in the chick, adipose tissue is responsible for approximately 30% of fatty acid synthesis, whereas liver, having only 1/3 the mass of adipose tissue (2.4% vs 6.8% of body wt. for liver and adipose tissue, respectively), accounts for the remaining 70%. In the mouse (2) and rat (3,4), adipose tissue is a much more important site of fatty acid synthesis, accounting for at least 50% and in some cases as much as 95% of the fatty acids synthesized. In the pigeon, on the other hand, adipose tissue has been estimated to account for only 4% of the fatty acids synthesized (7). Thus, the chicken appears to be intermediate to the mouse or rat, on the one hand, and the pigeon, on the other, with respect to the importance of adipose tissue as a site of fatty acid synthesis.

The method used in estimating the relative contribution of liver and adipose tissue to fatty acid synthesis has obvious limitations. The calculations are based on the assumption that the radioactive fatty acids found in each tissue represent synthesis in that tissue. This assumption appears reasonable, since the chicks used were in the fed state, and consequently mobilization of lipid should have been minimal. However, if liver is the major site of fatty acid synthesis in the chicken, as the present results and *in vitro* findings (6) suggest, translocation of radioactive lipid from liver to adipose tissue would be necessary. That this does occur is implied by the observed increase in plasma fatty acid radioactivity between 30 and 60 min after isotope administration. Therefore, some of the radioactive fatty acids found in adipose tissue undoubtedly originated in liver. This would have the effect of reducing even further the importance of adipose tissue relative to liver as a site of fatty acid synthesis. Therefore, the estimated value of 30% for the contribution of adipose tissue to total fatty acid synthesis in the chick represents a maximal value.

The finding that chick adipose tissue can readily utilize glucose-U-^{14}C for glyceride–glycerol synthesis is in accord with *in vitro* observations (6) and findings in the pigeon (7). The rapid decrease in adipose tissue glyceride–glycerol radioactivity (see Table I) suggests a high turnover rate for adipose tissue triglycerides in the chick. The present data do not permit an estimate of this rate of turnover, but it would appear to be substantial.

The results of the present studies show that the rate of hepatic lipogenesis in the chick is similar to that reported for the hen (10) and is considerably higher than that observed in the adult rooster (10). Thirty min after isotope administration, 0.3% and 0.7% of the administered glucose radioactivity was in liver fatty acids (Table I, Expts. I and II, respectively). When acetate-1-^{14}C was used (Table II), 2.6% of the administered dose was found in liver fatty acids 30 min after administration. This compares to values of 1.6 and 0.6% of administered acetate-^{14}C recovered as liver fatty acids in the hen and rooster, respectively, 30 min after administration (10). These data suggest that lipogenesis is high in the young growing chicken and in the male decreases with age as observed in the rat (11). In the hen, however, because of the necessity to produce lipid for egg formation lipogenesis remains high.

Summary. The ability of chicks to utilize glucose-U-^{14}C and acetate-1-^{14}C as substrates for lipid synthesis has been studied. Chicks readily incorporated glucose and acetate carbon into fatty acids and nonsaponifiable lipids of liver and adipose tissue. Glucose-U-^{14}C was also utilized for glyceride–glycerol formation. The relative importance of adipose tissue and liver as sites of fatty acid synthesis was estimated. Chick adipose tissue was found to be of minor importance as compared to liver, accounting for no more than 30% of total fatty acid synthesis.

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