Significance of Skin as a Site of Fatty Acid and Cholesterol Synthesis in the Chick[1] (36970)

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Studies have shown that skin is an active site of fatty acid and cholesterol synthesis in the human, rat, mouse and guinea pig (1–5). Ziboh, Dreize and Hsia (3) found that rat skin homogenates contained considerable amounts of some of the enzymes generally considered to be related to fatty acid synthesis namely malic enzyme, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. As in other tissues, lipid metabolism in skin is regulated. Thus starvation and alloxan diabetes diminished the capacity of human (6) and rat (7) skin to incorporate ^{14}C into lipids and refeeding or the administration of insulin, respectively, restored the impaired ability to synthesize lipids. A significant difference in capacity for lipid synthesis among various body sites has been shown by Griesemer and Thomas (2) and by Vroman, Nemecek and Hsia (8).

An earlier study from this laboratory (9) has shown that carcass (excluding liver and intestine) accounted for about one-third of the total cholesterol synthetic activity and about half of the total fatty acid synthetic activity in the chick. This indicated that extrahepatic fatty acid and cholesterol synthesis was significant in this species. The importance of skin as a site of fatty acid and cholesterol synthesis has not been studied in the chick. The experiments to be reported were designed to (a) determine fatty acid and cholesterol synthesis in skin samples, (b) directly compare lipogenesis in skin and other tissues namely liver and adipose tissue, and (c) evaluate the significance of skin as a site of fatty acid and cholesterol synthesis.

Materials and Methods. Male crossbred chicks (New Hampshire ♂ × Columbian ♀) were used for these studies. The chicks were fed a corn–soybean oil meal diet[3] and feed and water were supplied *ad libitum*.

In vitro *experiments*. Liver slices and skin specimens were obtained from chicks killed by cervical dislocation. The livers were rapidly removed and placed in cold saline (0.9% NaCl) and slices were prepared with a Stadie-Riggs hand microtome. The skin specimens were removed with scissors, immediately blotted free of blood, trimmed of fat and weighed. Adipose tissue, when taken, was removed from the breast and neck area. Incubations were carried out at 37° in 3 ml of Krebs–Ringer bicarbonate buffer [half amount of Ca^{2+} was added as recommended by Umbreit, Burris and Stauffer (11)] (pH 7.4), under 95% O_2 and 5% CO_2 in a metabolic shaker. Additions to the buffers are indicated in the tables of results. The procedures for isolating and determining the radioactivity of fatty acids and cholesterol have been described by Leveille (12). In these experiments saponification was in methanolic KOH, and the cholesterol digitonide was dissolved in methanol and an aliquot portion was taken for counting.

In vivo *experiments*. Each chick was given intravenously 5 μCi acetate-1-^{14}C contained in 0.5 ml of 0.9% NaCl solution. Fifteen minutes later the chicks were killed by cervical dislocation and the liver, intestine and skin were rapidly excised and weighed. The carcass was dried to constant weight at 100° in a forced-draft oven. The dried carcass was

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[3] For composition of the corn–soybean oil diet see Yeh and Leveille (10).

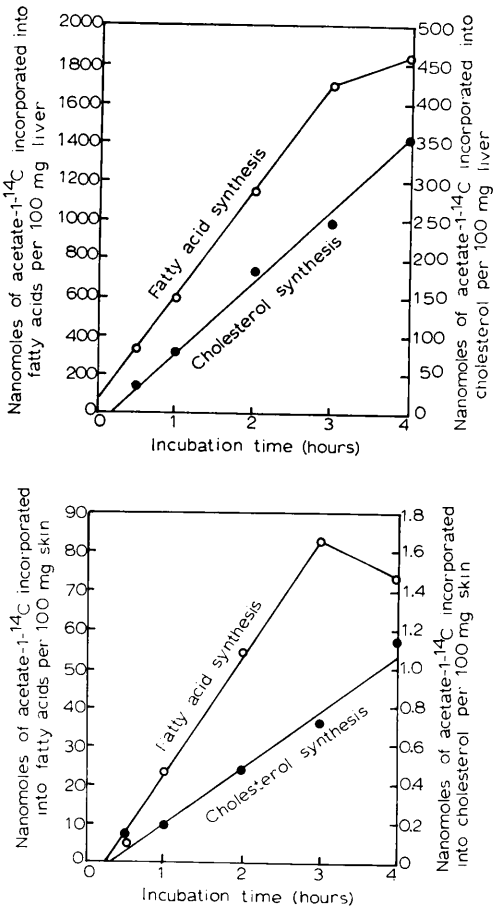

FIG. 1A. Effect of incubation time on *in vitro* fatty acid and cholesterol synthesis by chick liver. Buffer contained, per ml: 5 μmole glucose, 10 μmole sodium acetate, 0.15 μCi acetate-1-^{14}C and 0.1 unit insulin. Each point is the average of duplicate incubations. Tissue from 1 chick. (B) Effect of incubation time on *in vitro* fatty acid and cholesterol synthesis by chick skin. Buffer contained, per ml: 5 μmole glucose, 10 μmole sodium acetate, 0.15 μCi acetate-1-^{14}C and 0.1 unit insulin. Each point is the average of duplicate incubations. Tissue from 1 chick.

ground and a representative sample was taken for lipid analysis. The liver, intestine, skin and carcass lipids were isolated as previously described (13). The extracted tissue lipids were saponified in methanolic KOH, fatty acids and cholesterol were isolated and the radioactivity in each fraction was determined by the same procedure used for the *in vitro* studies.

Results. The *in vitro* incorporation of ^{14}C from acetate-1-^{14}C into fatty acids and cholesterol by chick liver and skin with time of incubation is shown in Fig. 1A and B. The rate of fatty acid and cholesterol synthesis was linear for at least 3 hr for both liver and skin. On the basis of these data, 3-hr incubations were carried out in subsequent experiments. The effect of acetate concentration on *in vitro* lipid synthesis by chick liver and skin was determined by incubating tissues with various concentrations of acetate in the presence of 5 m*M* glucose (Table I). These data indicate that the rate of fatty acid and cholesterol synthesis increased as the level of acetate in the medium increased from 5 to 10 m*M* and plateaued at 10 m*M* for both liver and skin. Therefore, 10 m*M* acetate was selected as the concentration for subsequent experiments. Results of an experiment conducted to compare acetate and glucose as substrates for fatty acid and cholesterol synthesis are presented in Table II. The addition of glucose to the incubation medium enhanced fatty acid synthesis from acetate-1-^{14}C in skin. However, the addition of glucose to the incubation medium did not stimulate fatty acid synthesis in liver nor cholesterol synthesis in liver or skin. Acetate in the presence of glucose was incorporated into fatty acids to a greater extent than glucose in either liver or skin and to a greater extent into cholesterol by liver. The molar incorporation of glucose and acetate into cholesterol by skin was similar. The experiment summarized in Table III compared fatty acid and cholesterol synthesis by liver, adipose tissue and by skin from different anatomical locations. It was found that fatty acid and cholesterol synthesis were much more active in liver than in adipose tissue or skin. Regardless of the site from which samples were taken, fatty acid but not cholesterol synthesis was higher in adipose tissue than in skin. There was some difference between skin from different anatomical locations with respect to fatty acid and cholesterol synthesis. Skin samples which included the feather tract had higher rates of fatty acid synthesis than comparable samples devoid of feather tract. Fatty acid synthesis by skin from the wing appeared to be lower, whereas cholesterol synthesis appeared

TABLE I. *In Vitro* Fatty Acid and Cholesterol Synthesis by Chick Liver and Skin Incubated with Different Concentrations of Acetate.[a]

Sodium acetate added (m*M*)	Fatty acid synthesis[b]		Cholesterol synthesis[b]	
	Liver	Skin	Liver	Skin
5	678[c]	34	162	0.9
10	1046	55	237	1.3
25	871	46	217	0.9
50	1137	60	151	1.5

[a] Buffer contained, per ml: 5 μmole glucose, 0.1 unit insulin, 0.15 μCi acetate-1-^{14}C for 5, 10, 25 m*M* sodium acetate added and 0.3 μCi acetate-1-^{14}C for 50 m*M* sodium acetate added and sodium acetate concentration as indicated.

[b] Nanomoles of acetate-1-^{14}C incorporated into fatty acids or cholesterol per 100 mg tissue in 3 hr.

[c] Average of triplicate incubations. Tissue from 1 chick.

to be higher, however these differences were small.

Data presented in Table IV indicate the contribution of different tissues of the chick to fatty acid and cholesterol synthesis when measured *in vivo*. The lipid content of the skin was 18% as compared to 5% for the liver, 4% for the intestine and 20% for the carcass. These percentages are expressed on a wet weight basis except for carcass which is expressed on a dry weight basis. The calculated contribution of skin to total fatty acid synthesis was about 7%, while skin accounted for about 6% of total cholesterol synthesis. The contribution of intestine to fatty acid synthesis was only 2% and for cholesterol synthesis 6%. This suggests that skin is a more important site of fatty acid synthesis than intestine but the two tissues are equally important insofar as cholesterol synthesis is concerned. After excluding liver, intestine and skin, the remaining carcass still contributed about 44% for total fatty acid synthesis and 24% for total cholesterol synthesis.

Discussion. The skin of the chick, like that of other animals, can synthesize fatty acid and cholesterol. This was demonstrated by both *in vitro* and *in vivo* experiments. Data presented in Fig. 1A and B indicate that the rate of *in vitro* lipid synthesis was linear over a 3-hr period. Thus it was possible to use incubation periods of 2–3 hr for studying the effect of various factors on rates of lipogene-

TABLE II. Comparison of Glucose vs Acetate as Substrates for *in Vitro* Fatty Acid and Cholesterol Synthesis by Chick Liver and Skin.

Substrate added	Fatty acid synthesis[a]		Cholesterol synthesis[a]	
	Skin	Liver	Skin	Liver
Acetate-1-^{14}C[b]	21 ± 3[c]	1486 ± 194	1.7 ± 0.4	174 ± 20
Acetate-1-^{14}C + glucose[d]	43 ± 5	1544 ± 261	2.2 ± 0.2	154 ± 13
Glucose-U-^{14}C + glucose[e]	29 ± 2	328 ± 105	2.3 ± 0.1	39 ± 16

[a] Nanomoles of acetate-1-^{14}C or glucose-U-^{14}C incorporated into fatty acids or cholesterol per 100 mg tissue in 3 hr.

[b] Buffer contained, per ml: 10 μmole sodium acetate, 0.15 μCi acetate-1-^{14}C and 0.1 unit insulin.

[c] Mean for 3 chicks ± SEM. Value for each chick is the average of duplicated incubations.

[d] Buffer contained, per ml: 5 μmole glucose, 10 μmole sodium acetate, 0.15 μCi acetate-1-^{14}C and 0.1 unit insulin.

[e] Buffer contained, per ml: 5 μmole glucose, 0.15 μCi glucose-U-^{14}C and 0.1 unit insulin.

TABLE III. *In Vitro* Fatty Acid and Cholesterol Synthesis by Liver, Adipose Tissue and Skin of the Chick.[a]

	Fatty acid synthesis[b]	Cholesterol synthesis[b]
Liver	1652 ± 324[c]	160 ± 27
Adipose tissue		
Breast region	71 ± 14	1.5 ± 0.6
Neck region	79 ± 12	0.3 ± 0.1
Skin		
Breast	46 ± 8	1.1 ± 0.2
Breast (feather tract)	56 ± 6	0.8 ± 0.3
Wing	21 ± 5	2.0 ± 0.5
Wing (feather tract)	36 ± 6	1.1 ± 0.3
Leg	44 ± 7	1.2 ± 0.2
Dorsal region	44 ± 6	0.9 ± 0.1

[a] Buffer contained, per ml: 5 μmole glucose, 10 μmole sodium acetate, 0.15 μCi acetate-1-^{14}C and 0.1 unit insulin. Average body weight of the chicks was 718 ± 42 g.

[b] Nanomoles of acetate-1-^{14}C incorporated into fatty acids or cholesterol per 100 mg tissue in 3 hr.

[c] Mean for 5 chicks ± SEM. Value of each chick is the average of duplicate incubations.

sis. The addition of glucose to the incubation medium enhanced fatty acid synthesis from acetate-1-^{14}C by chick skin. This finding is in agreement with previous studies by Griesemer and Thomas (1) with human skin and by Ziboh, Dreize and Hsia (3) and by Fulton and Hsia (14) with rat skin. Glucose stimulation of fatty acid synthesis has been attributed to its ability to serve as a source of α-glycerophosphate and to supply reducing equivalents to support fatty acid synthesis. Results presented in Table II indicate that acetate in the presence of glucose was a more effective precursor than glucose for fatty acid synthesis by chick skin, this was also found to be true for human skin (15). Presumably this is because acetate is a more immediate precursor of fatty acids than is glucose and does not pass through the regulatory steps of glycolysis prior to incorporation into fatty acids. Previous studies (2, 8) have shown that rates of lipogenesis for human skin vary significantly with body site. The results of a study to determine whether lipogenesis in chick skin varied with location indicated only moderate variations between sites. The results of this study indicate that the rate of lipogenesis in skin is markedly lower than in liver and significantly lower than in adipose tissue. However, since the total skin weight is about 3 times that of liver (Table IV), it

TABLE IV. *In Vivo* Fatty Acid and Cholesterol Synthesis in Different Tissues of Chicks.[a]

	Liver	Intestine	Skin	Carcass[b]
Tissue wt[c]	11.4 ± 0.4[d]	17.4 ± 0.6	33.6 ± 1.5	116.1 ± 2.6
Total lipid (%)[e]	4.98 ± 0.19	3.89 ± 0.44	18.48 ± 1.33	20.19 ± 0.28
Fatty acid synthesis[f] (% dose)	5.224 ± 1.213	0.235 ± 0.044	0.676 ± 0.153	4.392 ± 0.415
Fatty acid synthesis[g] (% total)	47.4 ± 7.0	2.2 ± 0.3	6.8 ± 2.0	43.6 ± 6.2
Cholesterol synthesis[f] (% dose)	0.270 ± 0.040	0.025 ± 0.005	0.054 ± 0.003	0.094 ± 0.011
Cholesterol synthesis[g] (% total)	64.3 ± 2.9	5.7 ± 0.9	6.4 ± 0.4	23.6 ± 3.0

[a] Average body weight of the chicks was 456 ± 9 g.

[b] Carcass excluding liver, intestine and skin.

[c] Values for liver, intestine and skin are wet weight, while carcass is dry weight.

[d] Mean for 5 chicks ± SEM.

[e] Percentage of wet weight for liver, intestine and skin, while dry weight for carcass.

[f] Each chick was given intravenously 0.5 ml of saline containing 5 μCi acetate-1-^{14}C and was killed 15 min after injection. Values represent percentage of the administered dose recovered in each tissue.

[g] Values represent percentage of total synthesis contributed by each tissue.

has been calculated that skin may contribute as much as 7% of the total fatty acids and 6% of the total cholesterol synthesized by the chick.

Summary. The significance of skin as a site of fatty acid and cholesterol synthesis has been studied in the chick. Both *in vitro* and *in vivo* experiments indicate that chick skin has the capacity to synthesize fatty acid and cholesterol. The addition of glucose to the incubation medium enhanced fatty acid synthesis but not cholesterol synthesis from acetate-1-^{14}C in chick skin. The rate of *in vitro* lipogenesis in skin is markedly lower than that in liver and slightly lower than that in adipose tissue. The estimated contribution of skin to total fatty acid synthesis in the intact chick was about 7%, while skin accounted for approximately 6% of total cholesterol synthesis.

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