Cutaneous lipogenesis: precursors utilized by guinea pig skin for lipid synthesis

VICTOR R. WHEATLEY, LEONARD T. HODGINS, WILLIAM M. COON, MUTUKUMARA KUMARASIRI, HAROLD BERENZWEIG, and JEFFREY M. FEINSTEIN

Department of Dermatology, New York University School of Medicine, New York 10016

ABSTRACT Cutaneous lipogenesis was studied, using a guinea pig ear slice incubation technique, for the following precursors: acetate, propionate, butyrate, glucose, pyruvate, lactate, succinate, citrate, and selected amino acids. Active lipogenesis was observed with short-chain fatty acids, glucose, pyruvate, lactate, and with the amino acids, alanine, leucine, and isoleucine. Glucose was shown to play an important role in cutaneous lipogenesis; it is a major precursor of lipid and the only compound able to stimulate lipogenesis. Its incorporation into lipid is unaffected by either insulin or epinephrine. The incorporation rates of glucose-1- and glucose-6-^{14}C were equal, suggesting the possibility that generation of NADPH by the pentose-phosphate pathway is minimal. Citrate, succinate, and pyruvate all failed to stimulate the incorporation of acetate; on the other hand, citrate, isocitrate, malate, malonate, and ATP caused inhibition of the incorporation of glucose. Significant incorporation of tritium from tritiated water was observed, and the order of magnitude suggests that it can be used as an independent assessment of the rate of cutaneous lipogenesis. Bicarbonate was not only able to stimulate the rate of incorporation of a variety of precursors but was also incorporated into fatty acids to a measurable extent. The mode of incorporation of propionate was unusual, since propionate-1-^{14}C was incorporated into fatty acids at more than double the rate for propionate-2-^{14}C, suggesting incorporation of the carboxyl carbon without the rest of the molecule. Mechanisms are suggested to account for the carbon dioxide fixation, but we are unable to completely explain the anomalous results for propionate.

SUPPLEMENTARY KEY WORDS glucose · fatty acid · tricarboxylic acid cycle · amino acids · regulation of lipogenesis · propionate

Lipogenesis occurs in the skin at two major sites, the Malpighian layer of the epidermis and the sebaceous gland, and it is more active in the latter site than in the former. The sebaceous cells undergo an enormous increase in size during lipogenesis; this is due to the lipid which is formed. Hence, while some precursors may be provided from materials already present in the cell when it differentiates, the majority must be brought to the cell by the circulating tissue fluids. Glucose is the obvious precursor; however, studies by Cruickshank, Trotter, and Cooper (1) indicate that in the absence of exogenous substrate the skin oxidizes protein and fat in preference to glucose. Furthermore, while it may be stated that in most tissues (2) all carbons of the fatty acids can be supplied by acetyl CoA, this is not true of skin. Odd-numbered and branched-chain fatty acids are important components of cutaneous lipids (3, 4). Precursors such as propionyl CoA, isobutyryl CoA, and methylmalonyl CoA must be used to form a part, at least, of such molecules. Confirmatory evidence is therefore required concerning the nature of the compounds used by skin to form lipids. Such compounds should be considered in terms of their ability to provide terminal or branched portions of the fatty acid chains and to provide the reductive hydrogens and acetyl CoA for elongation of the chain. In addition, some attention must be paid to their role as precursors for the sterols and other components of the nonsaponifiable fraction.

Previously we attempted to study this problem using the dog skin perfusion model (5) and achieved some degree of success in spite of the limitations of the method. We have now attempted to verify and extend our findings using a tissue slice incubation technique. The precursors we have studied include some representative examples of the following four classes of compounds: (*a*) short-

Abbreviations: TCA cycle, tricarboxylic acid cycle; TLC, thin-layer chromatography.

chain fatty acids, (*b*) glucose and products of glycolysis, (*c*) TCA cycle intermediates, and (*d*) amino acids.

Some observations have also been made on the roles played by water and carbon dioxide. The present study is intended as a preliminary broad-scope survey essential for the planning of more detailed studies of mechanisms and regulation. A brief report of a part of this work has been published (6).

MATERIALS AND METHODS

Materials

The ^{14}C-labeled compounds (listed in Table 3) were obtained from several sources. Compounds *a* through *r*, *v*, *w*, and *z* were from Amersham/Searle Corp., Des Plaines, Ill.; compounds *s*, *t*, *u*, *x*, and *y* were purchased from International Chemical and Nuclear Corp., Burbank, Calif.; compound *g* was also obtained from Tracerlab, Waltham, Mass.; and *d* and *e* were also obtained from Mallinckrodt Nuclear, St. Louis, Mo. The stated specific activities were 1–340 mCi/mmole, and assay data, provided by the suppliers, indicated purity of more than 98%. All compounds were used without further purification after a purity check using TLC or paper chromatography combined with radioautography, as described previously (5). Tritiated water was obtained from Volk Radiochemical Co., Burbank, Calif., and succinate-2,3-^{3}H was from New England Nuclear Corp., Boston, Mass.; the specific activities were 10 mCi/g and 94.7 mCi/mmole, respectively.

Unlabeled carrier compounds were obtained from J. T. Baker Chemical Co., Phillipsburg, N.J. (*a*); Fisher Scientific Co., Fair Lawn, N.J. (*f*, *g*, *l*, *m*, and *z*); Mann Research Laboratories, New York (*d*, *j*, *p* through *y*); and Sigma Chemical Co., St. Louis, Mo. (*n*). ATP was obtained from Calbiochem, Los Angeles, Calif., and glucose-6-phosphate, isocitrate, fumarate, malate, malonate, carnitine, insulin, and epinephrine were purchased from Sigma Chemical Co.

Standard Incubation Procedure

The guinea pigs used in these studies were nibblers which were fed Wayne Lab-blox (12% fat). Skin slices (10 mg) were cut free-hand from the outer tip of the ear as described by Cruickshank (7). They were incubated for 4 hr in 0.5 ml of Krebs-Ringer phosphate buffer (pH 7.4) containing 11.1 mM glucose, 0.057 mM streptomycin sulfate, and the labeled precursor at a concentration of 2 mM (except where otherwise specified) and a specific activity of 1 mCi/mmole. For convenience, incubations were performed in Cruickshank respirometers (Intectron Inc., Waltham, Mass.). The standard Cruickshank (7) respirometer and a modified incubation chamber used in these experiments are illustrated in Fig. 1; slices were placed in both wells of the respirometer and the manometer fluid was omitted. The respirometers were gassed with oxygen according to standard procedure and incubated without shaking (7). Incubations were performed in triplicate for each precursor, and control incubations, using acetate, were performed with the majority of experiments. One ear provided enough slices for 12 or 15 incubations to be performed at one time, and the slices were randomized throughout the series to compensate for slight variations from slice to slice. All data are based on two, or more, incubations of slices from different guinea pigs.

A limited number of incubations were performed using Krebs-Ringer bicarbonate buffer (pH 7.5 after equilibration) as medium and 5% carbon dioxide in oxygen as gaseous phase.

At the end of the incubation the tissue slices were removed from the incubation medium and dropped into 2 ml of M KOH in ethanol contained in a test tube fitted with a Teflon-lined screw-cap. Repeated examination of media after incubation failed to detect the presence of any radioactive lipid, hence saponification of the entire incubation system was unnecessary. The contents of the tubes were heated overnight at 70°C, cooled, diluted with water, and acidified with 10 N HCl, and the lipids were extracted with hexane. The hexane extract was washed repeatedly with a dilute solution of the appropriate unlabeled precursor and finally with water. The washed hexane extract was transferred to a clean test tube, and the solvent was evaporated in a stream of nitrogen, leaving the extracted lipids at the bottom of the test tube. A portion of the lipids was set aside for assay of total radioactivity and the remainder was fractionated as described below. No attempt was made to recover the glycerol liberated by saponification.

The lipids were separated into fatty acid and nonsaponifiable fractions by TLC on plates containing Silica Gel G (0.25 mm thick) prepared in this laboratory or purchased from Analtech, Wilmington, Del. The sample was applied to a TLC plate by means of a 2-cm Achával and Ellefson applicator (8) and the plate was developed until the solvent front traveled 5 cm. The developing solvent was chloroform–methanol–7 N ammonium hydroxide 83:15:2. The separation took only 5 min and gave satisfactory separation of nonsaponifiable material from fatty acids (Fig. 2). The bands were located by means of iodine vapor and, after allowing the iodine to volatilize, they were scraped from the plate directly into counting vials.

Measurement of Radioactivity

Scintillation fluid was used as previously described (5) except that Cab-O-Sil (Cabot Corp., Boston, Mass.)

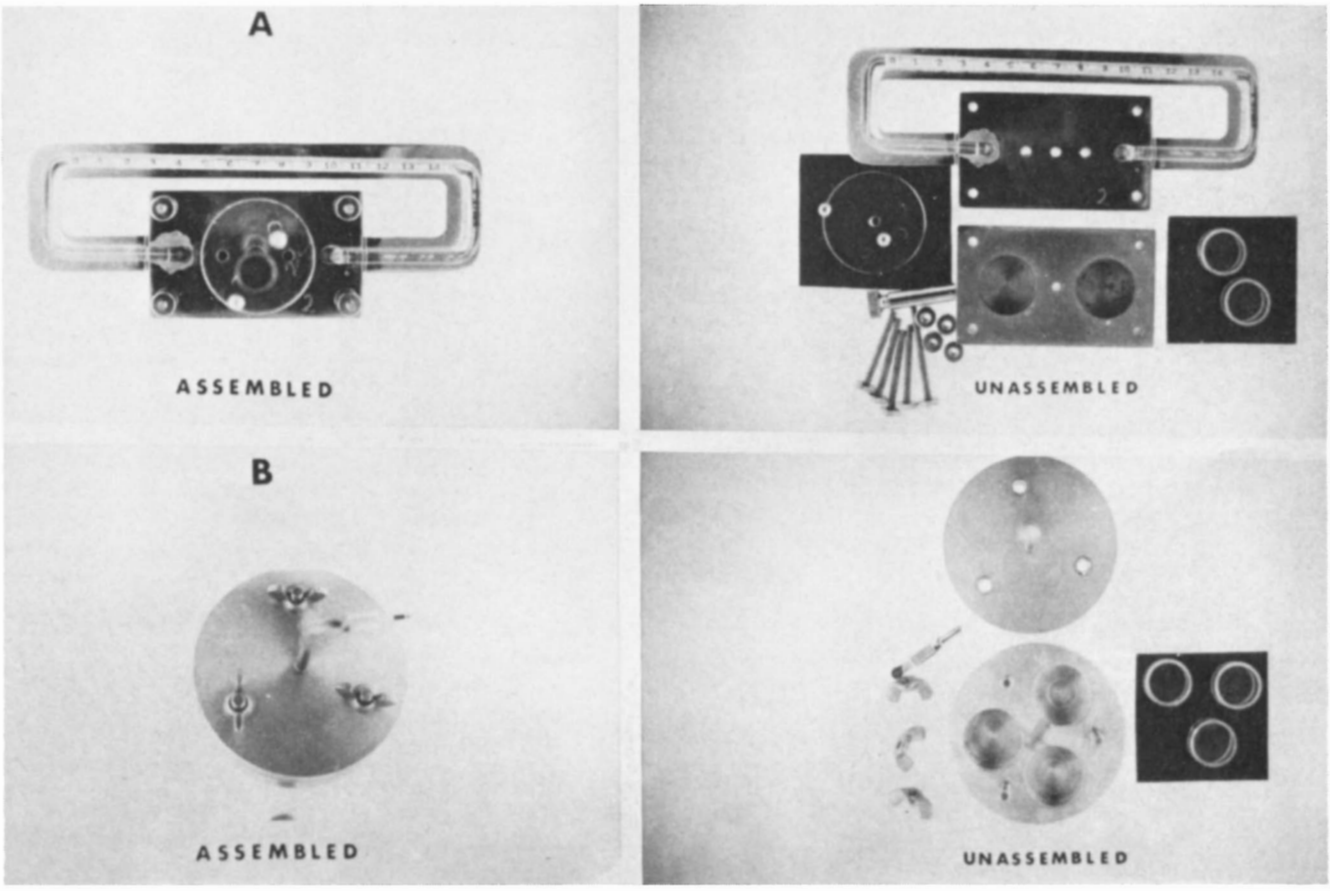

FIG. 1. Incubation chambers used in these experiments: (*A*), original Cruickshank (7) respirometer; (*B*), modified incubation chamber. The latter enables triplicate incubations to be performed simultaneously and removed from the water bath together.

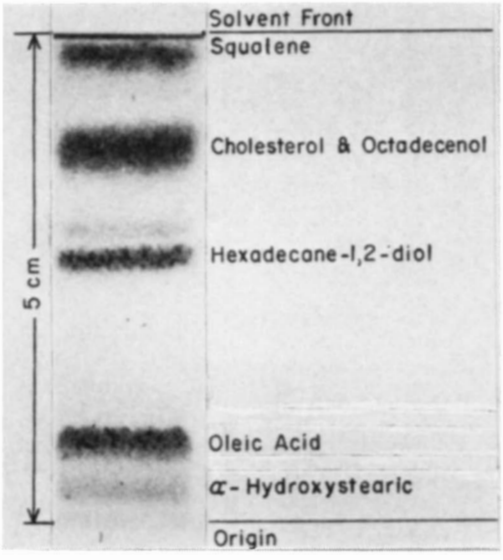

FIG. 2. Separation of fatty acids and nonsaponifiable material using TLC with chloroform–methanol–ammonium hydroxide as the developing solvent system. The band of the most polar compounds in the nonsaponifiable fraction, the alkane-1,2-diols, is widely separated from the fatty acid band. The latter can be considerably overloaded without affecting this separation.

was added to samples containing silica gel. Measurements were made in a Nuclear-Chicago "UNILUX" liquid scintillation spectrometer; the efficiency and quench corrections for ^{14}C were made by the channels ratio method (9); the corrections for ^{3}H were made by means of internal standards. In our system the efficiency for unquenched samples was 80% for ^{14}C and 24% for ^{3}H. The criteria of counting accuracy used were the same as those previously described (5).

Procedure for Fatty Acid Labeling Pattern Studies

In these experiments certain modifications of the standard procedure were made: 25 mg of tissue was used; the volume of media was kept at 0.5 ml; and 2 μCi of labeled compound was added to each cuvette with no unlabeled carrier added. After saponification the contents of the tubes were diluted with water and then extracted with hexane to remove the nonsaponifiable material. The aqueous phase was then acidified and extracted with hexane in the usual manner to recover the fatty acids. This separation was less complete than the TLC procedure but contaminating nonsaponifiable

matter was removed in the subsequent argentation TLC step. The fatty acids were esterified with freshly prepared ice-cold diazomethane and the unsaturated esters were removed by argentation TLC in the following manner. The esters were applied, as in standard procedure, to a silver nitrate-impregnated TLC plate (Mallinckrodt SilicAR TLC-7 suspended in 10% silver nitrate solution and spread to a thickness of 0.25 mm); the plates were developed in benzene until the solvent front reached 10 cm. Bands were located by ultraviolet light after the plates were lightly sprayed with 0.2% dichlorofluorescein in ethanol. The saturated ester band was scraped from the plate and the esters were recovered after extraction of the silica with chloroform in a micro-Soxhlet apparatus. The esters were then separated by gas–liquid chromatography as described below.

Other Analytical Procedures

Separation of dermis from epidermis was achieved by soaking the skin overnight in 2 M sodium thiocyanate (10), then gently pealing off the epidermis. In this procedure the sebaceous glands remain attached to the epidermis and can be removed from the dermis.

In experiments in which lipid classes were separated, the tissue, after incubation, was frozen in liquid nitrogen, pulverized, and then heated in chloroform–methanol 2:1. The lipid extract was washed as described by Folch, Lees, and Sloane Stanley (11). The extracted lipids were transferred to a TLC plate as in standard procedure, and the plate was developed in the three consecutive solvent systems described by Downing (12). The bands were located by means of iodine vapor and were scraped into counting vials.

In experiments requiring data on mass distribution of lipid classes, quantitative TLC–densitometry was performed exactly as described by Downing (12).

Gas–liquid chromatography was performed using a Glowall Chromalab apparatus modified in the following manner. The argon detector supplied, nominally a 1-cm Lovelock cell (13), departs from Lovelock's specifications and is nonlinear. The Teflon insulator was drilled to provide a second outlet (corresponding to *C* in Fig. 9 of Ref. 13) and the column effluent was connected to the bottom outlet of the cell as recommended by Lovelock for flow rates in excess of 20 ml/min. The modified detector, when operated at 700 volts, gave an acceptable linear response. Fraction collection was achieved by introducing a stream-splitter (a Swagelok 100-3-316 tee) in the detector oven with the collection outlet tube going through the lid of the oven. Collections were made manually using short lengths of glass tubing fitted with uniformly packed glass wool plugs (25 ± 1 mg). Analyses were performed on a 6-ft coiled glass column packed with SE-30-coated Gas-Chrom P (Applied Science Laboratories Inc., State College, Pa.) prepared in the following manner. The Gas-Chrom P (100–120 mesh) was pretreated as described by Haahti (14) and coated with the SE-30, using the method of Horning, Moscatelli, and Sweeley (15), to give a nominal coating of 1.5%. The column was programmed from 175°C to 300°C at 2°/min with argon as carrier gas at 40 psi; the flow rate was 60 ml/min (this varied somewhat as the temperature changed). Under the conditions used, recovery of injected palmitate-^{14}C was 60%.

RESULTS

Nature and Distribution of Lipids in Guinea Pig Skin

Guinea pig skin differs from that of other rodents in having a thick cellular epidermis with only a fine supporting framework of dermis; the ratio by weight of epidermis to dermis is 2.0 compared with 0.3 for the rat (16). As a result, the respiration and metabolic activity of guinea pig skin is appreciably higher than that of other mammals (16). Separation of dermis and epidermis of the skin from dog (thigh), mouse (whole body), and guinea pig (whole body) followed by analysis of lipid distribution emphasized this feature of the guinea pig. In mouse 87.7% and in dog 88.6% of the skin lipids were found in the dermis compared with 56.3% in the guinea pig. However, while the total metabolic activity of the guinea pig skin is higher than that of other rodents, this is not necessarily true for lipogenesis. In previous studies (17) acetate incorporation for guinea pig ear skin slices was compared with that of ear skin slices from other rodents with the following results: rats, almost double; gerbil, about equal; hampster, less than half. In subsequent experiments with mouse ear skin slices a rate of about five times that for the guinea pig was observed.

As determined by TLC–densitometry, approximately 80% of the guinea pig ear skin lipids are triglycerides (Table 1). This contrasts with the surface skin lipids, which contain no triglycerides (3, 18). The waxes and the free and esterified sterols, which are major components of the sebaceous lipids (comprising more than 90% of the total), are present in relatively small amounts in the ear lipids. A study of the incorporation of acetate-1,2-^{14}C into lipid classes by guinea pig ear skin slices (Table 1) suggests that a large portion of the triglycerides form an inert pool with a low rate of turnover, since only 30% of the incorporated activity is found in this fraction. Rapid turnover of epidermal and sebaceous lipids would be expected; hence, the inert triglyceride pool is probably in the dermis but its exact location remains obscure since, as reported elsewhere (17), the ear skin slices contain no dermal adipose cells.

TABLE 1 COMPOSITION OF GUINEA PIG EAR LIPIDS AND DISTRIBUTION OF ^{14}C IN EAR SKIN INCUBATED WITH ACETATE-1,2-^{14}C

	Mass	^{14}C
	%	
Hydrocarbons	0.0	3.2
Waxes and sterol esters	7.8	26.0
Triglycerides	79.3	30.0
Free fatty acids	6.9	14.0
Sterols (free)	4.0	12.9
Polar lipids	2.0	14.0

10-mg ear skin slices were incubated for 4 hr in 0.5 ml of phosphate buffer (pH 7.4) containing 11.1 mM glucose and 2 mM acetate (specific activity, 1 mCi/mmole).

As determined by gas–liquid chromatography, 51.9% of the total fatty acids were C_{18} unsaturated acids (with one, two, and three double bonds). Palmitic acid was the major saturated acid (30.4%), and there was 3.7% stearic acid. Branched-chain and odd-numbered acids were present (see Fig. 5), though in much smaller amounts than are found in the surface lipids (3). There were fatty acids with carbon numbers 14.7, 16.7, and 18.7, which corresponded in retention times to C_{15}, C_{17}, and C_{19} *anteiso*-acids; further identification was not attempted.

Incubation Conditions

Time course studies with representative precursors are shown in Fig. 3. In all cases an initial lag was observed during which the system came to equilibrium. After this lag, acetate and alanine showed essentially linear incorporation up to 4 hr, but glucose and pyruvate, at 2 mM, showed no increase after 3 hr. Glucose at 5 mM gave a curve which was concave upwards rather than linear, suggesting that a product-stimulated process was taking place. In the substrate concentration studies (Fig. 4), only the experiments with glucose showed a curve which began to plateau at 5 mM; the other substrates reached maximal incorporation at much higher concentrations. Final selection of substrate concentration had to be made arbitrarily, and 2 mM was chosen after consideration of the conditions used by investigators for tissues other than skin (19–23). In incubations with 2 mM glucose, the substrate was exhausted before the end of 4 hr, and in most studies with glucose a concentration of 5 mM was used. The selection of a 4-hr period for incubation was made for convenience.

Glucose is able to stimulate lipogenesis in certain tissues (24, 25) from a variety of precursors (23), but it is without effect in liver (26); this effect is believed to be related to the size of the endogenous glucose and glycogen pools in the tissue under study (27). In skin the endogenous pool is small, though not negligible (28). The effect of glucose on ear skin slice incorporation

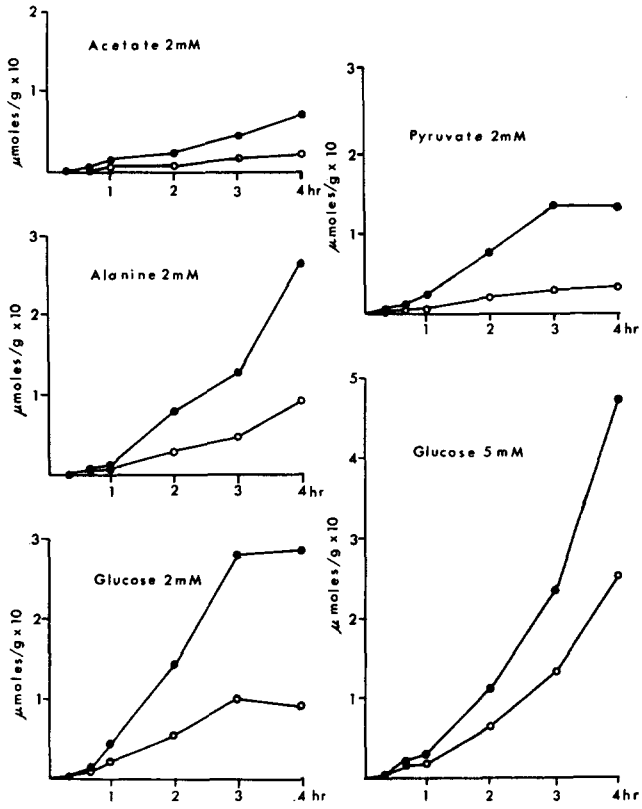

FIG. 3. Time course studies of the incorporation of acetate-1,2-^{14}C, pyruvate-2-^{14}C, alanine-U-^{14}C, and glucose-U-^{14}C into fatty acids (●) and nonsaponifiable material (○) by guinea pig ear slices. Incubations were performed with 10-mg ear skin slices as described in text, using a different animal for each compound.

rates was studied for a representative group of precursors (Table 2) and marked stimulation was observed in each case. It should be noted that there was significant incorporation of acetate in the absence of exogenous glucose, and there was even higher incorporation of pyruvate and lactate under these conditions. Since glucose produced constant stimulation of lipogenesis it was added to the medium in our standard procedure, and a concentration of 11.1 mM was selected on the basis of data from Cruickshank, Trotter, and Cooper (29).

Precursor Utilization

Incorporation rates for several precursors are presented in Table 3. Rates are expressed as nmoles of precursor incorporated into fatty acids and nonsaponifiable material per g wet tissue per hr of incubation (based on a 4-hr period) and are uncorrected for loss of radioactive carbon atoms during metabolism. We realize that these rates underestimate the true rate of substrate incorporation; however, we do not consider that this invalidates our interpretations. Since lipogenesis varied appreciably from animal to animal (17), the data have

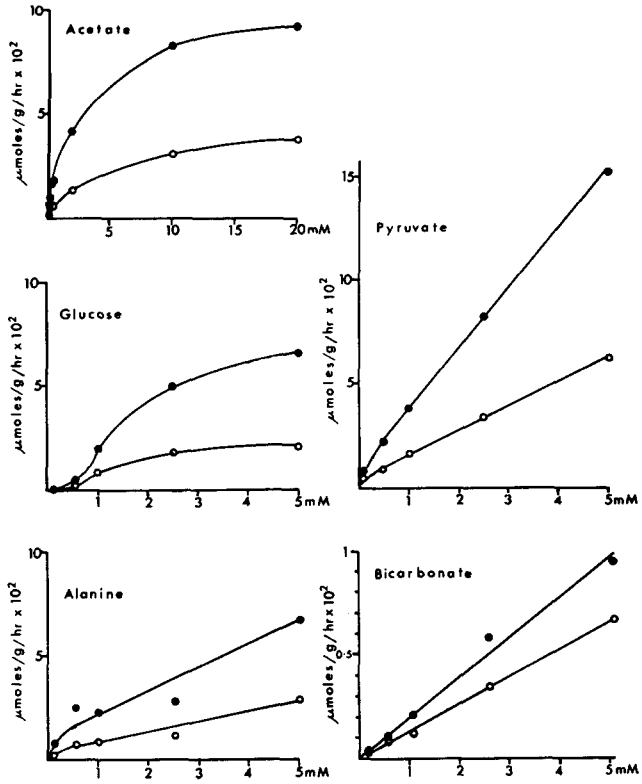

FIG. 4. Substrate concentration effects on the incorporation rates of acetate-1,2-^{14}C, pyruvate-2-^{14}C, alanine-U-^{14}C, bicarbonate-^{14}C (in the presence of 11.1 mM glucose), and glucose-U-^{14}C into fatty acids (●) and nonsaponifiable material (○) by 10-mg guinea pig ear skin slices. Incorporation rate is expressed as μmoles of precursor incorporated into fatty acids and nonsaponifiable material per g wet tissue per hr of incubation, based on a 4-hr incubation period. The slight changes in pH of the medium with increasing concentration of bicarbonate were not sufficient to cause an alteration in rate of lipogenesis. The rate scale for bicarbonate differs from the others by a factor of 10.

been presented in related groups with corresponding data for acetate control incubations at the beginning of each group. This method of presentation does not mean that experiments in each group were performed at the same time; indeed, in some cases an interval of almost 2 yr elapsed between individual experiments in a group. It was chosen for conciseness, and careful analysis of data from individual experiments shows that no inaccuracies or misrepresentations have thereby resulted. Comparison of these data with those in other tables may show inconsistencies because they do not necessarily represent the same groups of experiments. In all subsequent discussions, comparisons and tests for significance have been made on the data from individual experiments and not on the summarized data here presented.

Data on the rates of incorporation of some short-chain fatty acids are presented in the first group (Table 3, *a* through *f*). As anticipated, rapid incorporation of acetate was observed. The incorporation rates of acetate-1-^{14}C as compared with acetate-2-^{14}C were not equal, the latter being about 25% higher. Analysis of data from individual experiments showed this difference to be highly significant. Earlier investigators observed similar differences for other tissues. Feller (19) observed that acetate-2-^{14}C was incorporated into liver lipids at a rate 15% higher than acetate-1-^{14}C, but 50% lower in adipose tissue. The reason for these differences does not appear to have been resolved.

TABLE 2 EFFECT OF GLUCOSE ON THE INCORPORATION OF VARIOUS PRECURSORS INTO LIPIDS

Precursor	Glucose (11.1 mM)	Incorporation Rate into: Fatty Acids	Incorporation Rate into: Nonsaponifiable Material
		nmoles/g/hr	
Acetate-1,2-^{14}C	−	17.0 ± 3.9*	9.0 ± 2.1
	+	64.8 ± 2.3	11.1 ± 0.9
Pyruvate-2-^{14}C	−	29.3 ± 1.4	25.9 ± 3.8
	+	63.4 ± 4.5	46.2 ± 4.8
Lactate-2-^{14}C	−	29.3 ± 1.2	18.6 ± 1.4
	+	78.1 ± 21.4	30.4 ± 9.3
Isoleucine-U-^{14}C	−	2.5 ± 0.2	2.9 ± 0.2
	+	41.0 ± 5.4	16.6 ± 1.6
Leucine-U-^{14}C	−	5.5 ± 0.6	14.2 ± 1.0
	+	45.9 ± 4.4	28.7 ± 2.5
Bicarbonate-^{14}C	−	1.3 ± 0.1	3.7 ± 0.2
	+	5.0 ± 0.4	5.9 ± 0.3

* Mean ± SEM (n = 3). 10-mg ear skin slices were incubated for 4 hr in 0.5 ml of phosphate buffer (pH 7.4) containing 11.1 mM glucose and 2 mM labeled precursor (specific activity, 1 mCi/mmole). Rates assume linear incorporation and are not corrected for radioactive carbon lost during metabolism.

A relatively high rate of incorporation into fatty acids was observed for propionate, with much lower incorporation into nonsaponifiable material. The incorporation of propionate-1-^{14}C was more than double that for propionate-2-^{14}C; this unexpected observation was confirmed using labeled propionate obtained from different suppliers. As predicted theoretically and observed in other tissues (30–32), all three carbons of propionate should be incorporated as a unit or the acid decarboxylated and only C-2 and C-3 incorporated; hence the incorporation of the C-2-labeled compound should exceed that of the C-1-labeled. The incorporation rate of butyrate into skin lipids was observed to be much higher than that for acetate. The reverse is found in rat liver slices (33, 34).

More detailed studies were made of the incorporation of acetate-1,2-^{14}C and propionate-1-^{14}C into fatty acids. With the former, 41.2% of the incorporated activity was found in the unsaturated acids compared with 30.3% with the latter. The pattern of incorporation into saturated acids for both compounds was studied using gas–liquid chromatography. The incorporation patterns are shown in Fig. 5. Maximum incorporation

TABLE 3 INCORPORATION OF PRECURSORS INTO LIPIDS BY GUINEA PIG EAR SKIN SLICES

	Precursor	N	Rate of Incorporation into: Fatty Acids	Nonsaponifiable Material
			nmoles/g/hr	
(*a*)	Acetate-1,2-^{14}C	7	63.9 ± 5.2*	20.3 ± 3.4
(*b*)	Acetate-1-^{14}C	2	63.8 ± 4.8	28.7 ± 5.1
(*c*)	Acetate-2-^{14}C	2	81.6 ± 8.5	44.1 ± 6.7
(*d*)	Propionate-1-^{14}C	3	49.5 ± 5.7	6.5 ± 0.9
(*e*)	Propionate-2-^{14}C	3	21.1 ± 4.6	3.5 ± 0.5
(*f*)	Butyrate-1-^{14}C	2	147.2 ± 17.4	29.2 ± 1.7
	Acetate-1,2-^{14}C	6	62.9 ± 8.1	28.4 ± 3.9
(*g*)	D-Glucose-U-^{14}C	2	53.5 ± 8.9	16.6 ± 3.0
	D-Glucose-U-^{14}C†	5	101.3 ± 8.3	35.2 ± 3.5
(*h*)	D-Glucose-1-^{14}C†	2	214.9 ± 10.7	86.6 ± 10.9
(*i*)	D-Glucose-6-^{14}C†	2	195.4 ± 23.1	96.4 ± 17.0
(*j*)	Pyruvate-1-^{14}C	2	1.3 ± 0.3	1.6 ± 0.1
(*k*)	Pyruvate-2-^{14}C	2	104.0 ± 6.4	30.4 ± 2.8
(*l*)	D,L-Lactate-2-^{14}C	2	55.4 ± 9.3	28.9 ± 2.7
	Acetate-1,2-^{14}C	3	80.7 ± 12.0	31.0 ± 1.3
(*m*)	Citrate-1,5-^{14}C	2	4.6 ± 0.5	1.8 ± 0.1
(*n*)	Succinate-1,4-^{14}C	3	n.s.‡	n.s.
(*o*)	Succinate-2,3-^{14}C	3	7.0 ± 0.7	3.4 ± 0.2
	Succinate-2,3-^{3}H	2	7.8 ± 0.7	2.2 ± 0.3
	Acetate-1,2-^{14}C	11	119.5 ± 8.6	36.3 ± 2.4
(*p*)	L-Alanine-U-^{14}C	2	90.7 ± 11.8	18.8 ± 1.9
(*q*)	L-Aspartate-U-^{14}C	2	10.2 ± 0.6	2.5 ± 0.1
(*r*)	L-Glutamate-U-^{14}C	2	6.8 ± 1.7	2.1 ± 0.3
(*s*)	Glycine-1,2-^{14}C	2	1.2 ± 0.2	n.s.
(*t*)	L-Isoleucine-U-^{14}C	2	39.5 ± 1.6	6.9 ± 0.3
(*u*)	L-Leucine-U-^{14}C	2	70.4 ± 5.6	37.6 ± 5.0
(*v*)	L-Lysine-U-^{14}C	2	2.3 ± 0.1	n.s.
(*w*)	L-Methionine-(Me-^{14}C)	2	1.6 ± 0.3	n.s.
(*x*)	L-Phenylalanine-U-^{14}C	2	3.1 ± 0.2	1.8 ± 0.2
(*y*)	L-Valine-U-^{14}C	3	11.4 ± 1.7	2.9 ± 0.4
(*z*)	Bicarbonate-^{14}C	3	7.0 ± 0.6	7.0 ± 0.9
	Tritiated water	2	278.9 ± 27.6	131.6 ± 16.2

Incorporation rates were calculated from total uptake in 4 hr and assume a linear rate. No corrections were made for loss of radioactive carbon during metabolism (cf. correction for citrate used by Leveille and Hanson [70]). Sodium salts of all acids were used in these experiments. 10-mg skin slices were incubated for 4 hr with 0.5 ml of phosphate buffer (pH 7.4) containing unlabeled glucose and precursor (2 mM, specific activity 1 mCi/mmole).

* Mean ± SEM (n = 3N) for N groups of 3 incubations, a different animal being used for each group.

† The concentration of glucose in these incubations was 5 mM (0.4 mCi/mmole).

‡ Assessment of potential errors due to statistical error of counting and possible carry-over of traces of labeled precursor indicate that rates below 1.0 are not significant and have been designated n.s.

of acetate occurred in palmitic acid, with significant incorporation into other even-numbered acids. Incorporation into branched-chain and odd-numbered acids was also quite high. In contrast, the pattern for propionate-1-^{14}C shows maximal incorporation into odd-numbered acids. The even-numbered acids showed lowest incorporation while the branched-chain acids showed appreciable incorporation. The mode of incorporation into branched-chain acids has not been clarified, but a possible mechanism involving the introduction of a branching-unit via methylmalonyl CoA should be considered.

The second group of data relates to glucose and products of glycolysis (Table 3, compounds *g* though *l*). Glucose is rapidly incorporated into both fatty acids and nonsaponifiable material. After taking into account the effect of substrate concentration, it can be considered to be the most active precursor of lipid. Both glucose-1- and glucose-6-^{14}C were incorporated into fatty acids at equal rates; the difference between the figures

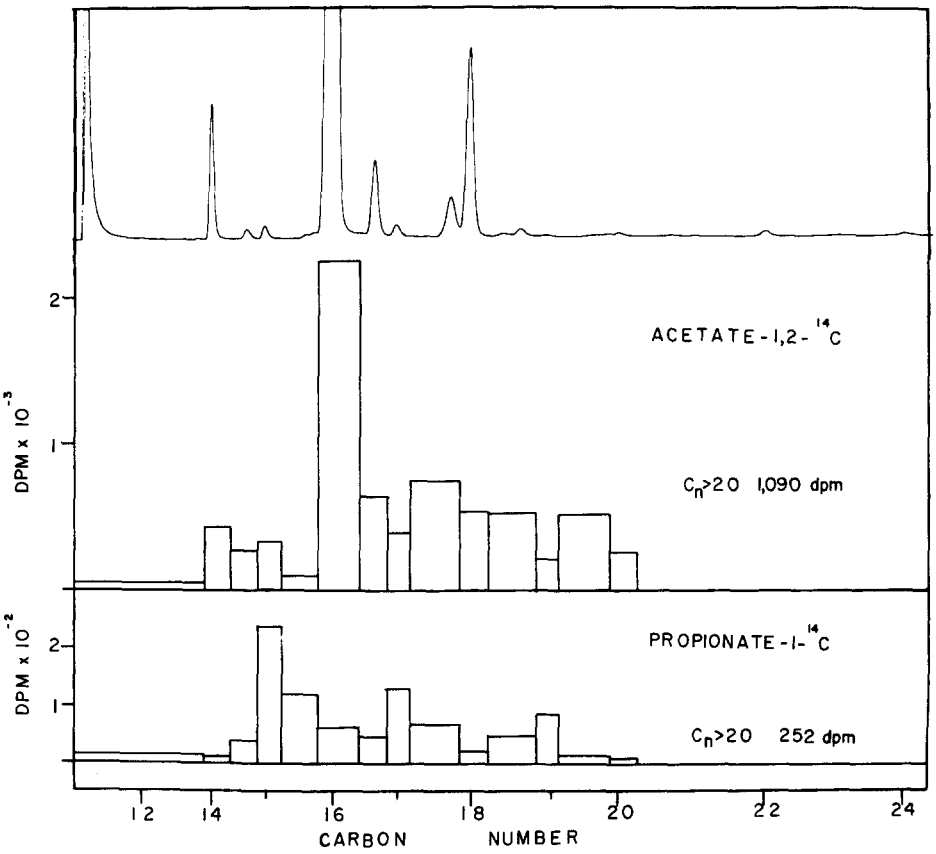

FIG. 5. Labeling patterns of saturated fatty acids isolated after incubating guinea pig ear skin slices with acetate-1,2-^{14}C and propionate-1-^{14}C as described in text. In the gas–liquid chromatogram at the top of the figure, the palmitate (C_{16}) peak has been run off scale to exaggerate minor peaks. For purposes of orientation, stearate (C_{18}) comprises 6.5% of the saturated fatty acids. Due to difficulties in detecting the low peaks beyond C_{20}, these were collected as a single fraction, and the counting data is presented numerically.

presented is not statistically significant. Pyruvate was incorporated at a high rate, comparable with that of glucose, with negligible incorporation of the carboxyl carbon. Appreciable incorporation of lactate was also observed; this was not unexpected in view of the significant lactate dehydrogenase (EC 1.1.1.27) activity in skin (35).

Citrate and succinate (Table 3, *m*, *n*, and *o*) showed low incorporation rates. Available data indicate that the uptake of citrate by skin cells is poor (36); hence, homogenates will be required for further studies of this metabolite. As observed with pyruvate, the carboxyl carbons of succinate are not incorporated into lipid. However, both succinate-2,3-^{3}H and succinate-2,3-^{14}C were incorporated at comparable rates, indicating the probability that two distinct mechanisms were operating. Whereat (37) observed incorporation of the hydrogens but not the carbons of succinate into fatty acids by aortic mitochondria.

Of the amino acids studied (Table 3, *p* through *y*), highest incorporation occurred with alanine, which could readily generate pyruvate. The branched-chain amino acids were incorporated at relatively high rates, valine being lowest. Aspartate and glutamate, which are metabolized via the TCA cycle, showed low incorporation. Incorporation of phenylalanine was even lower, and that of the remaining amino acids was negligible. In previous studies (38), examination of the fatty acids after perfusion with isoleucine showed highest incorporation into *anteiso*-C_{15} and C_{17} acids.[1]

Data on bicarbonate and tritiated water have been included here for completeness and will be discussed in detail in a later section. The measurable incorporation of bicarbonate into fatty acids by skin should be noted.

Action of Certain Metabolites, Hormones, and Cofactors

Limited studies were made of the effect of certain metabolites on the incorporation of acetate-1,2-^{14}C (Table 4). Of the compounds studied, only glucose stimulated incorporation. Pyruvate showed a slight but statistically insignificant effect, and citrate and

[1] Preliminary studies with our present system show similar results for guinea pig skin.

TABLE 4 EFFECT OF VARIOUS COMPOUNDS ON THE INCORPORATION OF ACETATE INTO FATTY ACIDS BY GUINEA PIG EAR SKIN SLICES*

	Concentration	Glucose†	Mean Incorporation Rate‡	*P* Value
	mM			
Citrate	10	−	69	0.10
Succinate	10	−	91	0 60
Glucose	10		381	<0.01
Pyruvate	10	−	126	0.40
ATP	5	+	116	0.40
Streptomycin	28.5	+	23	<0.01

* Results for nonsaponifiable material were similar to those for the fatty acids and have, therefore, not been presented. Incubation conditions as in Table 3.
† 11.1 mM.
‡ Percentage of control value.

TABLE 5 EFFECT OF CERTAIN HORMONES, METABOLITES, AND OTHER COMPOUNDS ON GLUCOSE INCORPORATION RATES INTO FATTY ACIDS BY GUINEA PIG EAR SLICES*

Substance	Concentration	Mean Incorporation Rate†	*P* Value
Insulin	1000 μU/ml	115	0.20
Epinephrine	0.2 μg/ml	114	0.40
Insulin and epinephrine	as above	99	>0.90
Glucose-6-phosphate	10 mM	133	0.20
Pyruvate	10 mM	29	<0.01
Acetate	10 mM	120	0.10
Citrate	10 mM	40	<0.01
Isocitrate	10 mM	59	0.02
Succinate	10 mM	92	0.20
Fumarate	10 mM	79	0.10
Malate	10 mM	9	<0.01
Malonate	10 mM	22	<0.01
Carnitine	5 mM	111	0.30
ATP	10 mM	1	<0.01

* Results for nonsaponifiable material were similar to those for the fatty acids and have, therefore, not been presented. Incubation conditions as in Table 3; 5 mM glucose was used throughout.
† Percentage of control value.

succinate were without effect; these three compounds stimulate incorporation in other tissues (39–43). ATP had no effect on incorporation, while streptomycin, at high concentrations, showed marked inhibition. The latter observation was made as a result of an error in medium preparation and was found to be true for a wide range of precursors in addition to acetate. At the concentration used in our standard medium streptomycin was without effect on lipogenesis.

More detailed studies were made with glucose as precursor (Table 5). Insulin and epinephrine, at concentrations at which the former causes marked stimulation and the latter inhibition in adipose tissue (44), were both without effect on skin. Glucose-6-phosphate, acetate, and carnitine showed a slight, but not statistically significant, stimulation of incorporation (for data on other tissues see 45, 46). ATP caused dramatic inhibition of lipogenesis from glucose even though it has no effect on the incorporation of acetate.

The slight reductions observed with succinate and fumarate were not statistically significant. Pyruvate, citrate, isocitrate, malate, and malonate all produced significant reduction in incorporation rate. This could be due to a dilution of pool size, enzyme inhibition, or a combination of both.

Role of Carbon Dioxide and Water

Bicarbonate causes marked increase of lipogenesis with most tissues (47) without being incorporated into fatty acids (19, 48, 49). Comparison of the incorporation rates in bicarbonate and phosphate buffers was made for several precursors (Table 6). In all cases significantly higher incorporation into fatty acids,

TABLE 6 EFFECT OF BICARBONATE ON THE INCORPORATION RATES OF VARIOUS PRECURSORS INTO LIPIDS BY GUINEA PIG EAR SKIN SLICES

Precursor	Buffer	Incorporation Rate into: Fatty Acids	Nonsaponifiable Material
		nmoles/g/hr	
Acetate-1,2-^{14}C	Phosphate	71.0 ± 7.6	21.1 ± 1.3
	Bicarbonate	126.7 ± 17.0	20.4 ± 3.8
D-Glucose-U-^{14}C*	Phosphate	100.2 ± 13.5	29.1 ± 4.2
	Bicarbonate	178.3 ± 6.5	37.2 ± 2.7
Pyruvate-2-^{14}C	Phosphate	110.1 ± 10.3	22.2 ± 1.5
	Bicarbonate	220.2 ± 11.4	25.0 ± 1.9
Citrate-1,5-^{14}C	Phosphate	5.5 ± 0.5	1.5 ± 0.1
	Bicarbonate	6.4 ± 0.6	1.1 ± 0.02
L-Isoleucine-U-^{14}C	Phosphate	37.4 ± 2.0	7.3 ± 0.3
	Bicarbonate	56.2 ± 2.8	7.1 ± 0.4

Incubation conditions as in Table 3.
* All substrates were 2 mM except glucose, which was 5 mM.

but not into nonsaponifiable materials, was observed with bicarbonate. The stimulatory effect was much lower than that observed in other tissues (46). On the other hand, unlike other tissues, measurable incorporation of bicarbonate into fatty acids was observed (Table 3). This incorporation was stimulated by glucose (Table 2) and increased with increasing substrate concentration (Fig. 3).

Significant incorporation of ^3H from tritiated water was observed for skin (Table 3). The rate of incorporation was of an order of magnitude comparable with that observed for glucose in this system. Jungas (50) has proposed the use of tritiated water as a reliable measure of the total rate of fatty acid synthesis in adipose tissue. Our results suggest that it can be used in this manner for skin. On the assumption that this was true, a study of the effects of acetate and glucose on the incorporation rates of tritiated water was undertaken (Table 7). In the absence of exogenous substrate a base rate of incorporation, representing lipogenesis from endogenous precursors, was observed. This rate was not increased when acetate was added in either tracer or higher concentrations; indeed, at 10 mM there was significant inhibition by acetate. Glucose, on the other hand, caused marked stimulation of the base rate, but when acetate was added in addition, no further stimulation occurred. The implication of these results is that acetate, even at high concentrations, acts as a tracer to label the acetyl CoA pool in this tissue and is unable to play the role of substrate for lipogenesis (the terms substrate and tracer are used here as defined by Emerson and Van Bruggen [51]).

TABLE 7 EFFECT OF ACETATE AND GLUCOSE ON THE INCORPORATION OF TRITIATED WATER INTO FATTY ACIDS BY GUINEA PIG EAR SKIN SLICES

Additions to Medium*	Incorporation Rate
	nmoles/g/hr
None	81.6 ± 15.5†
Acetate (25 μM)‡	60.0 ± 4.5 ($P = 0.20$)
Acetate (10 mM)	45.5 ± 5.6 ($P < 0.05$)
Glucose (10 mM)	299.4 ± 41.4
Glucose (10 mM) and acetate (10 mM)	284.6 ± 13.7

* Basic medium consisted of Krebs-Ringer phosphate buffer (pH 7.4) containing 0.057 mM streptomycin sulfate; 10 mg of ear skin slices were incubated for 4 hr in appropriate medium.

† Mean ± SEM (n = 3). Data represent results for ear slices from a single animal; comparable results, though with different rates, were observed on slices from a second animal.

‡ Figures in parentheses indicate final concentration in the incubation medium.

DISCUSSION

Cutaneous lipogenesis fulfills a physiological function not shared with other tissues, namely the synthesis of lipids for the specific biological role of maintaining the integrity of the integument. Teleological argument would suggest that it could not be subject to those mechanisms of regulation which would jeopardize this basic function. Two types of skin cells are known to undergo active lipogenesis, the keratinocyte and the sebocyte. The lipids formed by these cells perform separate physiological functions and are chemically different (5, 52). Hence, lipogenesis in the skin reflects the net result of two distinctly separate processes, each under the control of different mechanisms and each unique.

The subcutaneous fat is not a part of the skin proper, and it has a separate biological role. Unfortunately the line of demarcation is not always sharp, and in rodent skin, for example, relatively large numbers of adipocytes may be found in the lower dermis, above the muscle fascia, of skin from most sites of the body.[2] To avoid errors resulting from the concomitant presence of an unknown amount of adipose tissue cells, we selected for our studies skin from the tip of the guinea pig ear. The tip of the rodent ear consists of a central structure of cartilage covered by a layer of skin, without intervening adipose tissue. Histological examination of this skin shows it to be free of adipocytes. The large ears, thick epidermis (16), and placid disposition of the guinea pig make it an ideal experimental animal. Skin has proved refractory to metabolic studies because its fibrous nature makes the preparation of active homogenates difficult. Hence there is a paucity of reliable information concerning enzyme levels in whole skin and virtually no work with cell-free systems. This property of skin serves to advantage in tissue slice studies because the fibrous dermis acts as a sponge and penetration of precursors into the slice is rapid, permitting the incubation of very small pieces of tissue.

The present studies were planned as a broad-scope survey of cutaneous lipogenesis to pin-point those areas which merit more intensive study. They have provided convincing evidence of the important role played by glucose. It is almost certainly the major exogenous precursor of lipids and the only compound studied able to stimulate lipogenesis. Studies by other investigators (53, 54) have shown that glycogen storage in the sebaceous glands occurs prior to differentiation. Thus glycogenesis is actively functional; on the other hand, gluconeogenesis is not active in skin (36, 55). The skin cells appear to be freely permeable to glucose, and its uptake is not regulated by insulin. This conclusion

[2] The authors are indebted to Dr. William Montagna of the Oregon Regional Primate Center for discussions which helped to clarify our ideas on this subject.

is in agreement with the results of studies on human epidermis by Halprin, Ohkawara, and Adachi (56, 57).

The incorporation of glucose-1-^{14}C and glucose-6-^{14}C into fatty acids by skin appears to take place at equal rates. Using the method of calculation proposed by Abraham and Chaikoff (58), our data indicate a negligible contribution by the pentose-phosphate pathway. This is not consistent with the results of other workers (59, 60) who have demonstrated that this pathway functions in skin. There is, however, considerable doubt concerning its relative magnitude and, in a recent review, Mier (61) cites, as a reasonable estimate for human epidermis, that the pathway accounts for only 2% of total glucose oxidation. The latter estimate may be unduly conservative; nevertheless, all available evidence points to a much lower contribution by this pathway as compared with other tissues. Calculation shows that it would be unable to provide all the NADPH needed for lipogenesis, and a large deficit exists which must be generated by other mechanisms.

The increased incorporation of precursors in the presence of glucose represents a real increase in net lipogenesis, as shown by the studies with tritiated water. Both pyruvate and lactate are readily incorporated into fatty acids in the absence of exogenous glucose. On the other hand, pyruvate, without glucose, failed to stimulate acetate incorporation. Pyruvate can generate NADPH via the malate cycle, utilizing the mitochondrial NADH formed in generating acetyl CoA. It can also generate L-α-glycerophosphate by glycerogenesis (62) even if gluconeogenesis is inactive. (In skin, as in adipose tissue [63], *P*-enolpyruvate carboxylase [EC 4.2.2.32] is present, but hexosediphosphatase [EC 3.1.3.11] is not [64].) The glycerogenesis pathway from pyruvate would require a source of cytosolic NADH, and lactate could supply both this and the necessary pyruvate. Lactate incorporation, in the absence of exogenous glucose, however, is no greater than that of pyruvate. Either the NADH generated from lactate is not available for glycerogenesis or its rate of generation is too rapid and causes feed-back inhibition (65). Furthermore, the product-stimulation effect of glucose (see Fig. 3) could be due to the formation of L-α-glycerophosphate. All these data suggest a fine control of the generation of L-α-glycerophosphate with mutual regulation by synchronization with lipogenesis, coupled with the generation of cytosolic NADH.

Our observation of an incorporation of propionate-1-^{14}C into fatty acids that is double that for propionate-2-^{14}C is puzzling. Any metabolic pathway through succinyl CoA would involve partial or total randomization of C-2 and C-3 of propionate as a result of rearrangement of methylmalonyl CoA and reversibility of the enzymes involved (66). If in subsequent metabolism C-3 is lost, then the observed incorporation of propionate-2-^{14}C would be lower than theoretical. The observation that propionate-1-^{14}C was incorporated into even-numbered fatty acids (Fig. 5) is consistent with a pathway involving loss of C-3. Similar incorporation was observed by Katz and Kornblatt (31) for rat mammary gland, and they interpreted it in terms of the Vagelos and Earl (67) malonic acid pathway. They also observed, but were unable to explain, that propionate showed low incorporation into malate but high incorporation into glutamate and glutamine, whereas succinate showed high incorporation into malate only; all other data were consistent with the methylmalonyl CoA pathway. There is thus a hint, which should be further investigated, that a backwards pathway from succinyl CoA may be functioning in rodent skin and mammary gland. Nevertheless, even the most bizarre pathway through succinyl CoA will not explain the higher incorporation of propionate-1-^{14}C, while the incorporation of a C_3 unit will give equal incorporation of C-1 and C-2. Hence, other pathways must also operate which involve only C-1. A mechanism involving decarboxylation followed by fixation of the carbon dioxide so formed is feasible and consistent with our observation of measurable incorporation of bicarbonate into fatty acids. The latter rate, however, is too low to account for more than a part of the observed C-1 incorporation, while an alternate mechanism based on known metabolism of propionate does not appear feasible. We are unable to resolve the problem on the basis of present data.

The possibility that branched-chain fatty acids can be formed by skin from methylmalonyl CoA requires careful consideration. Such a mechanism is used to form the highly branched fatty acids of the uropygial gland (4) and may well be a common feature of all sebaceous glands. Since the formation of methylmalonyl CoA is mitochondrial (68), a mechanism of translocation is needed. Such a mechanism would, almost certainly, result in randomization of the carboxyl carbons and hence in the fixation of carbon dioxide as the methyl groups of the branched acids. This provides one feasible mechanism for carbon dioxide fixation; another mechanism could utilize methylcrotonyl CoA carboxylase (EC 6.4.1.4), where CO_2 would become the carboxyl carbon of the acetoacetate subsequently formed. This mechanism would require a continuous supply of leucine to generate the required methylcrotonyl CoA. This is possible in skin since both the keratinocyte and the sebocyte are dying cells; hence, proteolysis occurs readily in these cells. However, the β-hydroxy-β-methylglutaryl CoA formed first is an intermediate in sterol synthesis and we would anticipate higher incorporation into nonsaponifiable material than fatty acids;

this was not observed. Probably both the postulated mechanisms are active.

The results of our incorporation studies with amino acids indicate no basic differences from other tissues (23). There is a marked difference, however, between the data obtained with tissue slices and those obtained in our perfusion studies (5), suggesting an ability of skin in vivo to select the amino acids it requires for the biosynthesis of specific lipids. Measurable incorporation of both aspartate and glutamate is consistent with the operation in skin of the malate cycle and the citrate cleavage pathway. Other aspects of our data also suggest the functioning of both pathways and of the dicarboxylic acid shuttle, but confirmation by more rigorous studies is needed. High activity of isocitrate dehydrogenase (EC 1.1.1.42) in sebaceous glands has been observed by Cruickshank, Hershey, and Lewis (69) and may prove to be a major means of generating the balance of the NADPH needed by skin for lipogenesis. There are inconsistencies in some of our data which need clarifying, notably those concerning the response following the addition of certain metabolites. In conclusion, we consider the specific areas of cutaneous lipogenesis which merit vigorous investigation to be the metabolism of propionate, the modes of generating the supply of immediate precursors, and the relative contributions of the various pathways which can generate NADPH.

This work has been supported by Grant AM 11829 of the U.S. Public Health Service and by Training Grant AMO-5326. Summer Fellowships were provided for Mr. Berenzweig and Mr. Feinstein by the Dermatological Research Fund of this Department.

Manuscript received 23 October 1969 and in revised form 30 November 1970; accepted 22 January 1971.

References

1. Cruickshank, C. N. D., M. D. Trotter, and J. R. Cooper. 1962. A study of available substrates for the endogenous respiration of skin "in vitro." *J. Invest. Dermatol.* **39:** 175–178.
2. Foster, D. W., and J. Bloom. 1963. The synthesis of fatty acids by rat liver slices in tritiated water. *J. Biol. Chem.* **238:** 888–892.
3. Wheatley, V. R., and A. T. James. 1957. Studies of sebum. 7. The composition of the sebum of some common rodents. *Biochem. J.* **65:** 36–42.
4. Noble, R. E., R. L. Stjernholm, D. Mercier, and E. Lederer. 1963. Incorporation of propionic acid into a branched-chain fatty acid of the preen gland of the goose. *Nature (London).* **199:** 600–601.
5. Wheatley, V. R., G. Lipkin, and T. H. Woo. 1967. Lipogenesis from amino acids in perfused isolated dog skin. *J. Lipid Res.* **8:** 84–89.
6. Wheatley, V. R., L. T. Hodgins, H. Berenzweig, and W. M. Coon. 1969. Precursors for lipogenesis by skin. *Federation Proc.* **28:** 908. (Abstr.)
7. Cruickshank, C. N. D. 1954. Continuous observation of the respiration of skin *in vitro*. *Exp. Cell Res.* **7:** 374–380.
8. Achával, A., and R. D. Ellefson. 1966. Device for applying samples as a narrow band to thin-layer chromatograms. *J. Lipid Res.* **7:** 329–330.
9. Bush, E. T. 1963. General applicability of the channels ratio method of measuring liquid scintillation efficiencies. *Anal. Chem.* **35:** 1024–1029.
10. Selmanowitz, V. J., and V. R. Wheatley. 1968. The effects of hydrostatic pressure and lipid solvents on the water permeability of separated human epidermis. *Acta Dermato-Venereol.* **48:** 329–335.
11. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
12. Downing, D. T. 1968. Photodensitometry in the thin-layer chromatographic analysis of neutral lipids. *J. Chromatogr.* **38:** 91–99.
13. Lovelock, J. E. 1961. Ionization methods for the analysis of gases and vapors. *Anal. Chem.* **33:** 162–178.
14. Haahti, E. 1961. Major lipid constituents of human skin surface; with special reference to gas-chromatographic methods. *Scand. J. Clin. Lab. Invest.* **13(Suppl. 59):** 1–108.
15. Horning, M. G., E. G. Moscatelli, and C. C. Sweeley. 1959. Polyester liquid phases in gas–liquid chromatography. *Chem. Ind. (London).* 751–752.
16. Cruickshank, C. N. D., and J. R. Cooper, 1955. The contribution of epidermis and dermis to the respiration of the ear skin of the guinea pig, rat and mouse. *Exp. Cell Res.* **9:** 363–366.
17. Wheatley, V. R., L. T. Hodgins, and W. M. Coon. 1970. Cutaneous lipogenesis. I. Evaluation of model systems and the utilization of acetate, citrate and glucose as compared with other tissues. *J. Invest. Dermatol.* **54:** 288–297.
18. Nicolaides, N., H. C. Fu, and G. R. Rice. 1968. The skin surface lipids of man compared with those of eighteen species of animals. *J. Invest. Dermatol.* **51:** 83–89.
19. Feller, D. D. 1954. Metabolism of adipose tissue. I. Incorporation of acetate carbon into lipides by slices of adipose tissue. *J. Biol. Chem.* **206:** 171–180.
20. Feller, D. D., and E. Feist. 1959. Metabolism of adipose tissue: incorporation of isoleucine carbon into lipids by slices of adipose tissue. *J. Lipid Res.* **1:** 90–96.
21. Brady, R. O., F. D. W. Lukens, and S. Gurin. 1951. Hormonal influences upon the *in vitro* synthesis of radioactive fatty acids. *Science.* **113:** 413–415.
22. Madsen, J., S. Abraham, and I. L. Chaikoff. 1964. The conversion of glutamate carbon to fatty acid carbon via citrate. I. The influence of glucose in lactating rat mammary gland slices. *J. Biol. Chem.* **239:** 1305–1309.
23. Abraham, S., J. Madsen, and I. L. Chaikoff. 1964. The influence of glucose on amino acid carbon incorporation into protein, fatty acids and carbon dioxide by lactating rat mammary gland slices. *J. Biol. Chem.* **239:** 855–864.
24. Duncombe, W. G. 1968. Fatty acid synthesis in rat adipose tissue. Tracer effects *in vitro*. *Biochem. J.* **106:** 179–183.
25. Sørensen, M., and J. Madsen. 1967. Effect of glucose, pyruvate and α-ketoglutarate on acetate metabolism in rat mammary gland. *Proc. Soc. Exp. Biol. Med.* **126:** 643–648.
26. Hirsch, P. F., H. Baruch, and I. L. Chaikoff. 1954. The relation of glucose oxidation to lipogenesis in mammary tissue. *J. Biol. Chem.* **210:** 785–797.
27. Haugaard, E. S., and W. C. Stadie. 1952. Relation between

glycogen content and synthesis of fatty acids by rat liver. *J. Biol. Chem.* **199:** 741–744.
28. Adachi, K. 1961. Metabolism of glycogen in the skin and the effect of X-rays. *J. Invest. Dermatol.* **37:** 381–395.
29. Cruickshank, C. N. D., M. D. Trotter, and J. R. Cooper. 1957. Studies on carbohydrate metabolism of skin. *Biochem. J.* **66:** 285–289.
30. Feller, D. D., and E. Feist. 1957. Metabolism of adipose tissue. II. Incorporation of propionate carbon into lipides by slices of adipose tissue. *J. Biol. Chem.* **228:** 275–284.
31. Katz, J., and J. Kornblatt. 1962. Propionate metabolism by slices of mammary gland and liver of lactating rat. *J. Biol. Chem.* **237:** 2466–2473.
32. Cady, P., S. Abraham, and I. L. Chaikoff. 1963. Relative incorporation of various propionate carbons into fatty acids by lactating rat mammary gland. *Biochim. Biophys. Acta.* **70:** 118–131.
33. Brady, R. O., and S. Gurin. 1950. The biosynthesis of radioactive fatty acids and cholesterol. *J. Biol. Chem.* **186:** 461–469.
34. Elwood, J. C., A. Marcó, and J. T. Van Bruggen. 1960. Lipid metabolism in the diabetic rat. IV. Metabolism of acetate, acetoacetate and mevalonate *in vitro*. *J. Biol. Chem.* **235:** 573–577.
35. Halprin, K. M., and A. Ohkawara. 1966. Lactate production and lactate dehydrogenase in the human epidermis. *J. Invest. Dermatol.* **47:** 222–229.
36. Barron, E. S. G., J. Meyer, and Z. B. Miller. 1948. The metabolism of skin. Effect of vesicant agents. *J. Invest. Dermatol.* **11:** 97–118.
37. Whereat, A. F. 1965. Incorporation of tritium from succinate-2,3-H^3 into long-chain fatty acids by aortic mitochondria. *Proc. Soc. Exp. Biol. Med.* **118:** 888–892.
38. Wheatley, V. R., D. C. Chow, and F. D. Keenan. 1961. Studies of the lipids of dog skin. II. Observations on the lipid metabolism of perfused surviving dog skin. *J. Invest. Dermatol.* **36:** 237–239.
39. Rose, G., and B. Shapiro. 1955. Synthesis of fatty acids and glycogen by adipose tissue *in vitro*. *Biochim. Biophys. Acta.* **18:** 504–510.
40. Popják, G., and A. Tietz. 1954. Biosynthesis of fatty acids by slices and cell-free suspensions of mammary gland. *Biochem. J.* **56:** 46–54.
41. Foster, D. W., and B. Bloom. 1963. A reciprocal relationship between fatty acid and cholesterol biosynthesis. *Biochim. Biophys. Acta.* **70:** 341–343.
42. Winegrad, A. I., and A. E. Renold. 1958. Studies of rat adipose tissue *in vitro*. I. Effects of insulin on metabolism of glucose, pyruvate and acetate. *J. Biol. Chem.* **233:** 267–272.
43. Bloch, K., and W. Kramer. 1948. The effect of pyruvate and insulin on fatty acid synthesis *in vitro*. *J. Biol. Chem.* **173:** 811–812.
44. Flatt, J. P., and E. G. Ball. 1964. Studies on the metabolism of adipose tissue. XV. An evaluation of the major pathways of glucose catabolism as influenced by insulin and epinephrine. *J. Biol. Chem.* **239:** 675–685.
45. Flatt, J. P., and E. G. Ball. 1966. Studies on the metabolism of adipose tissue. XIX. An evaluation of the major pathways of glucose catabolism as influenced by acetate in the presence of insulin. *J Biol. Chem.* **241:** 2862–2869.
46. Franks, J. J., E. M. Riley, and K. J. Isselbacher. 1966. Synthesis of fatty acids by rat intestine *in vitro*. *Proc. Soc. Exp. Biol. Med.* **121:** 322–327.
47. Hirsch, J., and B. Goldrick. 1965. Metabolism of human adipose tissue in vitro. *In* Handbook of Physiology. Adipose Tissue. A. E. Renold and G. F. Cahill, Jr., editors. American Physiological Society, Washington, D.C. Section 5. 455–470.
48. Kneer, P., and E. G. Ball. 1968. Studies on the metabolism of adipose tissue. XXI. An evaluation of the major pathways of pyruvate metabolism. *J. Biol. Chem.* **243:** 2863–2870.
49. Gibson, G. N., E. B. Titchener, and S. J. Wakil. 1958. Mechanisms of fatty acid synthesis. V. Bicarbonate requirements for the synthesis of long-chain fatty acids. *Biochim. Biophys. Acta.* **30:** 376–383.
50. Jungas, R. L. 1968. Fatty acid synthesis in adipose tissue incubated in tritiated water. *Biochemistry.* **7:** 3708–3717.
51. Emerson, R. J., and J. T. Van Bruggen. 1958. Acetate metabolism: effects of tracer concentration. *Arch. Biochem. Biophys.* **77:** 467–477.
52. Nicolaides, N. 1965. Skin lipids. II. Lipid class composition of samples from various species and anatomical sites. *J. Amer. Oil Chem. Soc.* **42:** 691–702.
53. Montagna, W., H. B. Chase, and W. C. Lobitz, Jr. 1952. Histology and cytochemistry of human skin. II. Distribution of glycogen in epidermis, hair follicles, sebaceous glands and eccrine sweat glands. *Anat. Rec.* **114:** 231–248.
54. Henrikson, R. C. 1965. Glycogen and sebaceous gland transformation. *J. Invest. Dermatol.* **44:** 435–437.
55. Halprin, K. M., and D. C. Chow. 1961. Metabolic pathways in perfused dog skin. *J. Invest. Dermatol.* **36:** 431–439.
56. Halprin, K. M., A. Ohkawara, and K. Adachi. 1967. Glucose entry into the human epidermis. I. The concentration of glucose in the human epidermis. *J. Invest. Dermatol.* **49:** 559–560.
57. Halprin, K. M., and A. Ohkawara. 1967. Glucose entry into the human epidermis. II. The penetration of glucose into the human epidermis *in vitro*. *J. Invest. Dermatol.* **49:** 561–568.
58. Abraham, S., and I. L. Chaikoff. 1959. Glycolytic pathways and lipogenesis in mammary glands of lactating and non-lactating rats. *J. Biol. Chem.* **234:** 2246–2253.
59. Freinkel, R. K. 1960. Metabolism of glucose-^{14}C by human skin *in vitro*. *J. Invest. Dermatol.* **34:** 37–42.
60. Yardley, H. J., and G. Godfrey. 1963. Direct evidence for the hexose monophosphate pathway of glucose metabolism in skin. *Biochem. J.* **86:** 101–103.
61. Mier, P. D. 1969. The carbohydrate metabolism of skin. *Brit. J. Dermatol.* **18(Suppl. 2):** 14–17.
62. Ballard, F. J., R. W. Hanson, and G. A. Leveille. 1967. Phosphoenolpyruvate carboxykinase and the synthesis of glyceride-glycerol from pyruvate in adipose tissue. *J. Biol. Chem.* **242:** 2746–2750.
63. Weber, G., H. J. Hird, N. B. Stamm, and D. S. Wagle. 1965. Enzymes involved in carbohydrate metabolism in adipose tissue. *In* Handbook of Physiology. Adipose Tissue. A. E. Renold and G. F. Cahill, Jr., editors. American Physiological Society, Washington, D.C. Section 5. 225–237.
64. Halprin, K. M., and A. Ohkawara. 1966. Carbohydrate metabolism in psoriasis: an enzymatic study. *J. Invest. Dermatol.* **46:** 51–69.
65. Halperin, M. L., and B. H. Robinson. 1970. The role of the cytoplasmic redox potential in the control of fatty acid synthesis from glucose, pyruvate and lactate in white adipose tissue. *Biochem. J.* **116:** 235–240.
66. Sprecher, M., M. J. Clark, and D. B. Sprinson. 1966. The absolute configuration of methylmalonyl coenzyme A and

stereochemistry of the methylmalonyl CoA mutase reaction. *J. Biol. Chem.* **241:** 872–877.
67. Vagelos, P. R., and J. M. Earl. 1959. Propionic acid metabolism. III. β-Hydroxypropionyl coenzyme A and malonyl semialdehyde coenzyme A, intermediates in propionate oxidation by *Clostridium kluyveri*. *J. Biol. Chem.* **234:** 2272–2280.
68. Smith, R. M., and W. S. Osborne-White. 1965. Metabolism of propionate by sheep liver. Oxidation of propionate by homogenates. *Biochem. J.* **95:** 411–422.
69. Cruickshank, C. N. D., F. B. Hershey, and C. Lewis. 1958. Isocitric dehydrogenase activity of human epidermis. *J. Invest. Dermatol.* **30:** 33–37.
70. Leveille, G. A., and R. W. Hanson. 1966. Adaptive changes in enzyme activity and metabolic pathways in adipose tissue from meal-fed rats. *J. Lipid Res.* **7:** 46–55.