The Relative Significance of Acetate and Glucose as Precursors for Lipid Synthesis in Liver and Adipose Tissue from Ruminants

By R. W. HANSON AND F. J. BALLARD

*Fels Research Institute and the Department of Biochemistry,
Temple University Medical School, Philadelphia, Pa. 19140, U.S.A.*

(Received 20 February 1967)

1. The incorporation of labelled glucose into lipid by liver slices from sheep and cows is considerably less than that by liver slices from the rat, although oxidation to carbon dioxide occurs to a similar extent. ATP citrate lyase and NADP malate dehydrogenase are inactive in both sheep and cow liver but active in rat liver. The absence of the citrate-cleavage pathway of lipogenesis in ruminant liver has been confirmed by the negligible amounts of C-3 of aspartate incorporated into fatty acids. 2. Considerable amounts of [^{14}C]acetate are incorporated into fatty acids and non-saponifiable lipid in rat and ruminant liver. Acetyl-CoA synthetase, the initial enzyme in the metabolism of acetate, has a high activity in liver from rat and ruminants. 3. In adipose tissue from ruminants more acetate than glucose is converted into lipids, whereas the converse is true in rat adipose tissue. The greater incorporation of [^{14}C]acetate into fatty acids in adipose tissue from the ruminant as compared with the non-ruminant may be caused, in part, by the higher activity of acetyl-CoA synthetase activity in the ruminant. 4. The results suggest that, in both liver and adipose tissue from ruminants, acetate is a more important source of lipid than glucose. 5. Two enzymes of the hexose monophosphate shunt, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, are active in both tissues and from the three species.

In non-ruminant animals, glucose derived from dietary carbohydrate is the main precursor for the synthesis of lipids (Winegrad, 1965; Dole, 1965). The situation is less clear in ruminants, where dietary carbohydrate is converted into various short-chain intermediates before absorption. The available glucose in ruminants is synthesized in the liver and kidney and the obvious premium on this source of carbohydrate suggests that the products of rumen metabolism such as acetate and butyrate are the major precursors for lipogenesis in these animals (Annison & Lindsay, 1961; Holdsworth, Neville, Nader, Jarrett & Filsell, 1964; Kleiber, Smith, Black, Brown & Tolbert, 1952). The purpose of the present work was to determine the relative importance of glucose and acetate as substrates for lipogenesis in liver and adipose tissue in the cow and sheep. We have also sought to establish the presence of a number of enzymes involved in the synthesis of lipids and to determine the pattern of their activity in order to clarify the overall pathway of lipogenesis in ruminant as compared with non-ruminant animals.

MATERIALS AND METHODS

Chemicals. [$\text{U-}^{14}\text{C}$]Glucose, [$1\text{-}^{14}\text{C}$]acetate and $\text{NaH}^{14}\text{CO}_3$ were purchased from New England Nuclear Corp., Boston,

Mass., U.S.A.; DL-[$3\text{-}^{14}\text{C}$]aspartate and DL-[$4\text{-}^{14}\text{C}$]aspartate were from Volk Radiochemical Corp., Skokie, Ill., U.S.A.; NAD, NADP, NADH_2 , ATP, ITP, CoA and acetyl-CoA were from P-L Laboratories, Milwaukee, Wis., U.S.A.; citrate synthase (EC 4.1.3.7) and NAD malate dehydrogenase (EC 1.1.1.37) were from Boehringer Corporation, New York, N.Y., U.S.A. Malate, glucose 6-phosphate, 6-phosphogluconate and GSH were purchased from Sigma Chemical Co., St Louis, Mo., U.S.A. The highly purified glucagon-free insulin used in this study was a gift from Dr O. K. Behrens, Eli Lilly Laboratories, Indianapolis, Ind., U.S.A.

Animals. The sheep used in this study were 5–7-year-old cross-bred females, and the cows were 3–5-year-old female Holsteins in various stages of gestation and lactation. The rats were males of the Wistar strain, 350–400 g., obtained from Carworth Farms Co., New City, N.Y., U.S.A. All animals were fed *ad libitum*; rats on Purina Lab Chow; sheep were fed on lucerne hay with mixed grain supplements twice daily; cows were grazed on a mixed grass pasture and also had mixed grain supplements twice daily. The sheep were killed by electrocution and the cows with a captive bolt fired into the brain. The animals were bled and sections of mesenteric adipose tissue and liver were removed. The adipose tissue was kept at $35\text{--}40^\circ$ in 0.9% NaCl before use and the liver in chilled 0.9% NaCl. Rats were killed by a sharp blow to the head and the epididymal fat pads and liver were removed and handled similarly to those from the sheep and cow.

Incubation studies. Liver was sliced as outlined by

Ballard & Oliver (1964a) and rinsed in Krebs-Ringer bicarbonate buffer, pH 7.4. Both the liver slices and sections of adipose tissue were weighed and approx. 200 mg. transferred to Erlenmeyer flasks (25 ml.) containing 5.0 ml. of Krebs-Ringer bicarbonate buffer, pH 7.4 (Umbreit, Burris & Stauffer, 1959). The flasks were equipped with a plastic bucket containing a 2 cm. x 3 cm. piece of filter paper. A rubber stopper served both as a cap to close the flasks and to hold the plastic buckets suspended into the flasks above the buffer. The substrates were dissolved in the buffer at the following concentrations: [^{14}C]acetate, 50 mM (2.5 μC); [^{14}C]glucose, 50 mM (2.5 μC). Insulin (0.1 unit) was added with the glucose. In the experiment with L-aspartate (5 mM), 2.5 μC of either [^{14}C]aspartate or [^{14}C]aspartate was added together with 0.1 unit of insulin and 25 μmoles of glucose (5 mM). The tissue was incubated at 37° for 3 hr. in an atmosphere of $\text{O}_2 + \text{CO}_2$ (95:5) in a shaking water bath.

At the end of the incubation, 0.15 ml. of 40% (w/v) KOH was injected through the plasma stopper on to the filter paper and 0.5 ml. of $\text{N-H}_2\text{SO}_4$ into the buffer to ensure complete liberation of CO_2 . After shaking for 30 min. the tissue was removed, rinsed in 0.9% NaCl and transferred to 10 ml. of chloroform-methanol (2:1, v/v). The lipid was extracted and the radioactivity measurements were performed as described by Hanson (1965). The filter paper was removed from the flask, placed in a liquid scintillation vial and allowed to dry in air overnight. The dried paper was aligned at the bottom of the vial and the radioactive bicarbonate measured directly by the method of Buhler (1962). All radioactivity determinations were performed on a Nuclear-Chicago liquid-scintillation spectrometer. The efficiency of the system as determined by channels ratio was 60–70% and all values were corrected to 100% efficiency on this basis.

In preliminary experiments with glucose and acetate at concentrations lower than 50 mM we found lower amounts of incorporated radioactivity. At the higher concentrations, the incorporation of radioactivity was approximately linear throughout the 3 hr. incubation period.

The expected labelling in fatty acid from [^{14}C]aspartate and [^{14}C]aspartate has been described in detail (Leveille & Hanson, 1966). C-4 of aspartate would not be incorporated into fatty acids by any known pathway. On the other hand, C-3 of aspartate is incorporated into fatty acids via the citrate-cleavage pathway (Scheme 1). The labelled intermediates would be: [^{14}C]oxaloacetate, [^{14}C]malate, [^{14}C]pyruvate, [^{14}C]acetyl-CoA, [^{14}C]citrate and [^{14}C]acetyl-CoA. This is the simplest pathway and is based on the assumption that aspartate is converted into oxaloacetate in the cell supernatant and that label in oxaloacetate does not randomize through a symmetrical intermediate. The more complex pathways would also result in incorporation of label from C-3 of aspartate into fatty acids, whereas no label from C-4 of aspartate would be incorporated (Leveille & Hanson, 1966).

Preparation of tissue for enzyme measurements. Adipose tissue and liver were homogenized with a coaxial homogenizer in buffered iso-osmotic sucrose solution (Henning, Stumpf, Ohly & Seubert, 1966). As adipose tissue from cow and sheep could not be homogenized in this manner if it was previously chilled, this tissue was kept at between 35–40° up to this stage. The homogenates were centrifuged at 100 000g for 30 min. in a Spinco model L ultracentrifuge

at 2°. The supernatant was used for the assay of glucose 6-phosphate dehydrogenase (EC 1.1.1.49), 6-phosphogluconate dehydrogenase (EC 1.1.1.44), NADP malate dehydrogenase (EC 1.1.1.40), ATP citrate lyase (EC 4.1.3.8), citrate synthase, pyruvate carboxylase (EC 6.4.1.1) and acetyl-CoA synthetase (EC 6.2.1.1). The particulate fraction was suspended in buffered sucrose, freeze-dried, resuspended in water and used for the assay of citrate synthase and pyruvate carboxylase.

Assay of glucose 6-phosphate dehydrogenase. This enzyme was assayed as described by Kornberg & Horecker (1955).

Assay of 6-phosphogluconate dehydrogenase. This enzyme was assayed as described by Horecker & Smyrniotis (1955).

Assay of NADP malate dehydrogenase. The spectrophotometric assay as described by Ochoa (1955b) was used with the final concentrations of reagents as reported by Ballard & Hanson (1967a).

Assay of ATP citrate lyase. Oxaloacetate formation from citrate, CoA and ATP was measured as the oxidation of NADH_2 by coupling with NAD malate dehydrogenase according to Srere (1962) as modified by Ballard & Hanson (1967a).

Assay of citrate synthase. The equilibrium of the NAD malate dehydrogenase reaction strongly favours the formation of malate. Oxaloacetate is only formed from malate and NAD when a coupling system for the removal of oxaloacetate is present. With added acetyl-CoA the activity of citrate synthase is measured by the rate of formation of oxaloacetate and thus NADH_2 . This method is essentially that of Ochoa (1955a).

The final concentrations of reactants in the assay performed at 37° were: tris (adjusted to pH 8 with 2N-HCl), 150 mM; sodium malate, 6.6 mM; acetyl-CoA, 0.5 mM; NAD, 1.5 mM; excess of NAD malate dehydrogenase and tissue extract in a volume of 1.5 ml. The change in E_{340} with acetyl-CoA omitted was subtracted as a blank from the complete system.

Assay of acetyl-CoA synthetase. The principle of this assay is similar to that described above for citrate synthase, with the activity of acetyl-CoA synthetase limiting the production of NADH. This method is similar to that described by Beinert *et al.* (1951). The final concentrations of reactants at 37° were: tris (adjusted to pH 9.0 with 2N-HCl), 100 mM; potassium acetate, 10 mM; NAD, 1.5 mM; MgCl_2 , 10 mM; potassium ATP, 5 mM; potassium malate, 10 mM; GSH, 10 mM; CoA, 0.5 mM; excess of citrate synthase and NAD malate dehydrogenase. The E_{340} change with CoA omitted was used as the blank and subtracted from the complete system. This blank, or one with either ATP or acetate omitted, was approx. 20% of the test in most assays.

Assay of pyruvate carboxylase. The assay is in principle similar to that used by Utter & Keech (1960) and measures the fixation of ^{14}C -labelled bicarbonate in the presence of ATP, pyruvate, MgCl_2 , acetyl-CoA and enzyme. The exact composition of the reaction mixture was reported by Ballard & Hanson (1967b).

RESULTS

Comparison of lipogenesis in ruminant and non-ruminant liver. The rates of conversion of acetate and glucose into carbon dioxide, fatty acid and non-saponifiable lipid by liver slices from rats,

Table 1. *Glucose and acetate utilization by liver slices from various animals*

Values are the means of determinations on four animals $\pm$ s.e.m. Liver slices were incubated in 5.0 ml. of Krebs-Ringer bicarbonate buffer, pH 7.4, containing either 50 mM-[U- 14 C]glucose or 50 mM-[1- 14 C]acetate. The metabolites measured were isolated as outlined in the Materials and Methods section.

Metabolite measured	Substrate	Substrate converted (μ moles/g. wet wt. of liver/3 hr.)		
		Rat	Sheep	Cow
CO ₂	Glucose	5.66 $\pm$ 0.46	1.88 $\pm$ 0.49	0.72 $\pm$ 0.18
	Acetate	11.90 $\pm$ 0.36	9.87 $\pm$ 0.72	8.70 $\pm$ 1.9
Fatty acid	Glucose	1.55 $\pm$ 0.28	0.024 $\pm$ 0.006	0.007 $\pm$ 0.003
	Acetate	1.47 $\pm$ 0.36	0.20 $\pm$ 0.08	0.08 $\pm$ 0.009
Non-saponifiable lipid	Glucose	0.13 $\pm$ 0.02	0.02 $\pm$ 0.005	0.008 $\pm$ 0.004
	Acetate	0.16 $\pm$ 0.01	0.14 $\pm$ 0.04	0.14 $\pm$ 0.05

Scheme 1. Citrate-cleavage pathway and its relationships to lipogenesis from glucose and acetate. The enzymes of the pathway are as follows: (1) pyruvate carboxylase; (2) pyruvate dehydrogenase; (3) citrate synthase (mitochondrial); (4) ATP citrate lyase; (5) NAD malate dehydrogenase; (6) NADP malate dehydrogenase; (7) citrate synthase (cytoplasmic); (8) acetyl-CoA synthetase.

sheep and cows are presented in Table 1. Both substrate incorporation values and enzyme rates were calculated initially on both a nitrogen and a wet-weight basis. Since both methods of expressing the values gave similar results in a comparison

between species only the wet-weight values are included. Glucose is oxidized to carbon dioxide at a much higher rate in rat than in sheep or cow liver, whereas acetate is oxidized at approximately similar rates by liver slices from all three species.

Table 2. *Activities of enzymes involved in lipid synthesis in liver*

Supernatant and particulate fractions of liver homogenates were assayed for certain enzyme activities as described in the Materials and Methods section. All activities are expressed as $\mu\text{moles/min./g. wet wt. of liver}$ at 37° . Values are the means $\pm$ s.e.m. of determinations on the numbers of animals given in parentheses. Values taken from Ballard & Hanson (1967a) are indicated by an asterisk.

Pathway	Enzyme	Activities in liver		
		Rat	Sheep	Cow
Hexose monophosphate pathway	Glucose 6-phosphate dehydrogenase	4.66 ± 0.86 (6)	1.59 ± 0.29 (5)	1.86 ± 0.29 (5)
	6-Phosphogluconate dehydrogenase	10.50 ± 1.02 (6)	6.59 ± 2.65 (5)	2.85 ± 0.41 (5)
Citrate-cleavage pathway	Citrate synthase, particulate	6.61 ± 0.54 (5)	2.81 ± 0.33 (5)	1.69 ± 0.50 (4)
	ATP citrate lyase	1.84 ± 0.41 (6)*	0.025 ± 0.009 (5)	0.051 ± 0.02 (5)
	NADP malate dehydrogenase	1.97 ± 0.36 (6)*	0.004 ± 0.002 (5)	0.009 ± 0.008 (5)
Acetate metabolism	Acetyl-CoA synthetase	1.25 ± 0.32 (5)	0.69 ± 0.10 (5)	0.63 ± 0.03 (4)
	Citrate synthase, supernatant	0.59 ± 0.02 (5)	0.28 ± 0.10 (5)	0.51 ± 0.10 (4)

Table 3. *Incorporation of [3- ^{14}C]aspartate and [4- ^{14}C]aspartate into lipid and carbon dioxide by liver slices from sheep and rats*

Liver slices were incubated in 5 ml. of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 5 mm-aspartate (2.5 μC), 5 mm-glucose and 0.1 unit of insulin. The products were measured as described in the Materials and Methods section. Values are the means $\pm$ s.e.m. of determinations of four animals.

Labelled substrate	Product measured	Aspartate incorporated ($\mu\text{moles/g. wet wt. of liver/3 hr.}$)	
		Rat	Sheep
[3- ^{14}C]Aspartate	CO_2	1078 ± 75	542 ± 78
	Fatty acid	151 ± 29	0.91 ± 0.05
	Non-saponifiable lipid	21.1 ± 2.8	3.31 ± 0.43
[4- ^{14}C]Aspartate	CO_2	2960 ± 332	1390 ± 18
	Fatty acid	0.19 ± 0.03	0.24 ± 0.04
	Non-saponifiable lipid	0.23 ± 0.04	0.22 ± 0.01

In ruminant liver lipid synthesis from both glucose and acetate occurs at a much lower rate than in rat liver and acetate is far more effective than glucose as a precursor. Although the incorporation of acetate into non-saponifiable lipids was similar in the three species, the amount of glucose conversion in rat liver was much greater than in the livers of the sheep and cow.

The activities of all enzymes measured were higher in rat liver than in sheep or cow liver (Table 2). Glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in rat liver were 1.5–4-fold more active than in ruminant liver. Of the three enzymes involved in the citrate-cleavage pathway (Scheme 1), ATP citrate lyase and NADP malate dehydrogenase activities were extremely low in sheep and cow liver, being 20–500-fold less than the corresponding activity in rat liver. Particulate citrate synthase in rat liver had 2–3 times the activity in ruminant liver. Acetyl-CoA syn-

thetase and citrate synthase activities in the supernatant in ruminant liver were from half to almost equal to the corresponding activities in rat liver.

Both the glucose incorporation data and the enzyme activity measurements indicate that the citrate-cleavage pathway is not functional in ruminant liver. To further clarify the role of this pathway, incorporation experiments were performed with specifically labelled aspartate as substrate. The results are shown in Table 3. More of C-4 of aspartate than C-3 was oxidized to carbon dioxide in both rat and sheep liver slices, and no label from [4- ^{14}C]aspartate was found in either fatty acid or non-saponifiable lipid. C-3 of aspartate was incorporated into non-saponifiable lipid in liver from both animals. C-3 of aspartate was incorporated into fatty acids at a high rate in rat liver, whereas its incorporation was negligible into fatty acids in sheep liver.

Table 4. *Glucose and acetate utilization by adipose tissue from various animals*

Values are the means $\pm$ S.E.M. of determinations on four animals. The tissue was incubated in 5.0 ml. of Krebs-Ringer bicarbonate buffer, pH 7.4, containing either 50 mM-glucose or 50 mM-acetate. The metabolites measured were isolated as outlined in the Materials and Methods section.

Metabolite measured	Substrate	Substrate converted (μ moles/g. wet wt. of tissue/3 hr.)		
		Rat	Sheep	Cow
CO ₂	Glucose	2.40 $\pm$ 0.68	0.40 $\pm$ 0.08	0.35 $\pm$ 0.10
	Acetate	0.70 $\pm$ 0.26	1.19 $\pm$ 0.20	0.98 $\pm$ 0.12
Fatty acid	Glucose	0.56 $\pm$ 0.10	0.08 $\pm$ 0.02	0.06 $\pm$ 0.02
	Acetate	0.03 $\pm$ 0.009	0.25 $\pm$ 0.11	0.22 $\pm$ 0.04
Non-saponifiable lipid	Glucose	0.49 $\pm$ 0.07	0.07 $\pm$ 0.02	0.08 $\pm$ 0.02
	Acetate	0.02 $\pm$ 0.003	0.23 $\pm$ 0.05	0.30 $\pm$ 0.12

Table 5. *Activities of enzymes involved in lipid synthesis in adipose tissue*

Supernatant and particulate fractions of adipose tissue homogenates were assayed for the activity of certain enzymes as described in the Materials and Methods section. All activities are expressed as μ moles/min./g. wet wt. of tissue at 37°. Values are the means $\pm$ S.E.M. of determinations on the numbers of animals given in parentheses. Values taken from Ballard & Hanson (1967b) are indicated by an asterisk.

Pathway	Enzyme	Activities in adipose tissue		
		Rat	Sheep	Cow
Hexose monophosphate pathway	Glucose 6-phosphate dehydrogenase	770 $\pm$ 218 (6)	389 $\pm$ 49 (4)	1120 $\pm$ 414 (4)
	6-Phosphogluconate dehydrogenase	376 $\pm$ 36 (6)	215 $\pm$ 26 (4)	523 $\pm$ 137 (4)
Citrate-cleavage pathway	Citrate synthase, particulate	600 $\pm$ 55 (6)	63 $\pm$ 14 (4)	36 $\pm$ 5.5 (4)
	ATP citrate lyase	52.8 $\pm$ 8.4 (4)	9.0 $\pm$ 1.7 (4)	24 $\pm$ 10.0 (4)
	NADP malate dehydrogenase	323 $\pm$ 40 (4)	67 $\pm$ 14 (4)	63 $\pm$ 14.9 (4)
	Pyruvate carboxylase, supernatant	79 $\pm$ 10 (5)*	1.0 $\pm$ 0.2 (4)	7.8 $\pm$ 1.5 (4)
	Pyruvate carboxylase, particulate	140 $\pm$ 23 (6)*	3.1 $\pm$ 0.6 (4)	4.8 $\pm$ 1.3 (4)
Acetate metabolism	Acetyl-CoA synthetase	39 $\pm$ 6 (4)	125 $\pm$ 29 (4)	75 $\pm$ 13.8 (4)
	Citrate synthase, supernatant	374 $\pm$ 83 (6)	88 $\pm$ 10 (4)	87 $\pm$ 11.0 (4)

Comparison of lipogenesis in ruminant and non-ruminant adipose tissue. Glucose is converted into carbon dioxide in rat adipose tissue more rapidly than in that of sheep and cow (Table 4). On the other hand, the oxidation of acetate is similar in adipose tissue from all three species. Differences are more pronounced when the incorporation rates into fatty acid and non-saponifiable lipids are compared. Thus fatty acids and non-saponifiable lipids are formed from glucose at much higher rates in rat adipose tissue than in sheep or cow adipose tissue, whereas from acetate the rates of labelling of fatty acids and non-saponifiable lipids are much lower in the rat than in the ruminants.

The activities of enzymes involved in lipid synthesis in rat and ruminant adipose tissue are shown in Table 5. The activities of the two enzymes

of the hexose monophosphate pathway are quite variable among the three species, with the lowest activities in sheep adipose tissue. Included in the group of enzymes that we have considered as part of the citrate-cleavage pathway are soluble and particulate pyruvate carboxylase. Low activities in ruminant adipose tissue were found for particulate citrate synthase, ATP citrate lyase, NADP malate dehydrogenase and especially for both forms of pyruvate carboxylase. Acetyl-CoA synthetase activity was higher in adipose tissue from the sheep and cow than in the rat. Less soluble citrate synthase activity was present in ruminant adipose tissue than in adipose tissue from the rat.

Effect of glucose plus insulin on acetate utilization. The addition of glucose and insulin to the Krebs-Ringer bicarbonate buffer containing 50 mM-acetate

Table 6. *Effect of glucose plus insulin on acetate utilization by adipose tissue and liver from various animals*

Values are the means of three experiments. Tissues were incubated in 5.0 ml. of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 50 mM-acetate, 2.5 μ C of [14 C]acetate, 50 mM-glucose and 0.1 unit of insulin. The lipid and CO₂ were isolated and measured as described in the Materials and Methods section.

Animal	Substrate	Acetate converted (μ moles/g. wet wt. of tissue/3hr.)			
		Liver		Adipose tissue	
		CO ₂	Lipid	CO ₂	Lipid
Cow	Acetate	8.77	0.24	0.89	0.52
	Acetate + glucose + insulin	13.24	0.22	1.85	1.80
Sheep	Acetate	8.89	0.13	1.01	0.34
	Acetate + glucose + insulin	11.60	0.24	1.64	1.96
Rat	Acetate	11.90	1.63	0.70	0.05
	Acetate + glucose + insulin	7.73	1.70	1.34	0.90

generally increased the utilization of acetate in both liver and adipose tissue (Table 6). In the former the increase in carbon dioxide and lipid labelling was only slight, but in adipose tissue the increase in the rate of lipid synthesis from acetate was 3-fold in the cow, 6-fold in the sheep and 20-fold in the rat. A 1.5–2.5-fold increase was found in the production of carbon dioxide under the same conditions.

DISCUSSION

Lipogenesis in the rat occurs via a series of reactions involving the conversion of glucose into pyruvate, followed by the intramitochondrial formation of acetyl-CoA. This cannot diffuse into the cytoplasm for fatty acid synthesis (Spencer & Lowenstein, 1962). To circumvent this mitochondrial barrier, acetyl-CoA condenses with oxaloacetate to form citrate, which passes into the cytoplasm where the citrate is cleaved by ATP citrate lyase to form extramitochondrial oxaloacetate and acetyl-CoA for lipogenesis (Spencer & Lowenstein, 1962; Srere, 1959). It has been further suggested by Young, Shrago & Lardy (1964) and Wise & Ball (1964) that the oxaloacetate generated by citrate cleavage could be converted into malate via NAD malate dehydrogenase and then into pyruvate via NADP malate dehydrogenase in the cytoplasm. An important aspect of this scheme is the formation of NADPH₂ for the reductive synthesis of fatty acids, via the coupling of the two malate dehydrogenases. Support for the functioning of this citrate-cleavage pathway was provided by Leveille & Hanson (1966), who demonstrated the conversion of aspartate-derived oxaloacetate into fatty acids in rat adipose tissue by a pathway involving citrate cleavage. To complete the citrate-cleavage pathway a source of intramitochondrial oxaloacetate for citrate formation is required. This

is most probably supplied by the action of pyruvate carboxylase, which is present in the mitochondria of liver and adipose-tissue cells (Ballard & Hanson, 1967b). This overall pathway is summarized in Scheme 1.

In the present work it has been shown that, in the absence of glucose, the utilization of acetate for lipid synthesis in sheep and cow adipose tissue is 10 times that in rat adipose tissue. This incorporation is increased by addition of glucose and insulin to the incubation medium to a smaller extent with ruminant than with rat adipose tissue. The initial enzymic step in the conversion of acetate into fatty acids is the formation of acetyl-CoA via the acetyl-CoA synthetase reaction. This enzyme is more active in both the cow and sheep than in rat adipose tissue (Table 5). Acetyl-CoA synthetase allows the ruminant to form acetyl-CoA directly in the cytoplasm of the fat cell, thus bypassing the need for citrate to move acetyl-CoA across the mitochondrial membrane. In this paper we have reported low activities of the enzymes of the citrate-cleavage pathway and low rates of glucose conversion into lipid in adipose tissue in ruminants as compared with the non-ruminant. Although Khachadurian, Adrouni & Yacoubian (1966) have reported a substantial uptake of glucose by tail fat of the sheep, the need to conserve glucose for important processes such as brain metabolism (McClymont & Setchell, 1956), lactation (Kronfeld, Mayer, Robertson & Raggi, 1963) and for foetal glucose (Kronfeld, 1958) might be expected to limit severely the glucose available for lipid synthesis.

Balmain, Folley & Glascock (1954) studied the utilization of glucose and acetate for lipogenesis by mammary-gland slices from lactating rats and sheep. The rat slices used 8 times more glucose and 6 times less acetate than did slices from the liver of lactating sheep. In a separate study, the same

authors (Balmain, Folley & Glascock, 1953) reported that the addition of glucose plus insulin to sheep mammary-gland slices did not affect the rate of acetate conversion into fatty acids, though producing a 50–100-fold increase in the rat.

In the present study there were several important differences in the pattern of lipogenesis in the liver of the rat as compared with that of the cow and sheep. Liver slices from the cow and sheep used acetate to a far greater extent than glucose, but the overall rate of fatty acid synthesis from both substrates was lower in ruminant than in rat liver. On the other hand, the synthesis of non-saponifiable lipid from acetate was the same in ruminant and non-ruminant liver. The larger proportion of acetate carbon incorporated into non-saponifiable lipid in cow and sheep liver as compared with rat liver most likely meets the demands of these animals for cholesterol for steroid biosynthesis and cell-membrane formation. It may be argued that, as the amounts of short-chain fatty acids derived from the rumen are already substantial, the synthesis of fatty acids in the liver to support the respiration of tissues such as muscle would not be advantageous in the ruminant. An additional explanation for the low rates of fatty acid synthesis from glucose in ruminants may also be found in the dynamics of glucose utilization in the gut. Since dietary glucose is not adsorbed into the portal circulation (Roe, Bergman & Kon, 1966), it seems unlikely that net uptake of glucose into the ruminant liver ever occurs under physiological conditions (see also Barker, 1961). Further support for this suggestion is based on the finding that the high- K_m hepatic glucokinase is absent from ruminant liver (Ballard & Oliver, 1964b, 1965).

The block in glucose conversion into fatty acids in ruminant liver has been shown to be in the citrate-cleavage pathway by the use of differentially labelled aspartate and specifically pinpointed as an absence of both ATP citrate lyase and NADP malate dehydrogenase. The absence of ATP citrate lyase from goat liver was previously shown by Hardwick (1965, 1966).

The synthesis of long-chain fatty acids requires large amounts of NADPH₂ (Langdon, 1957; Wakil, Porter & Gibson, 1957; Flatt & Ball, 1964). Glucose, the normal substrate for lipogenesis in non-ruminant tissues, will also provide NADPH₂ both by oxidation through the hexose monophosphate pathway and by decarboxylation of malate (from citrate cleavage) to pyruvate (see Scheme 1). Since acetate is utilized for lipogenesis in ruminant tissue in the absence of added glucose, the source of reducing equivalents to support lipogenesis is in question. Under physiological conditions enough glucose may be metabolized via the hexose monophosphate pathway to supply adequate

amounts of NADPH₂. High activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are found in adipose tissue and liver from the three species studied in this paper.

The authors thank Dr David Kronfeld, Dr Klaus Hubben and the staff of the University of Pennsylvania Veterinary School for making their facilities available to us, and acknowledge helpful discussions with Dr Kronfeld and Dr Sidney Weinhouse during this study. This work was performed with the excellent technical assistance of Miss Linda Fisher, Miss Joan Krassenstein and Miss Marlene Orenberg. This work was supported by Grants CA-07174 and Am-05487 from the U.S. Public Health Service.

REFERENCES

- Annisson, E. F. & Lindsay, D. B. (1961). *Biochem. J.* **78**, 777.
 Ballard, F. J. & Oliver, I. T. (1964a). *Biochem. J.* **90**, 261.
 Ballard, F. J. & Oliver, I. T. (1964b). *Biochem. J.* **92**, 131.
 Ballard, F. J. & Oliver, I. T. (1965). *Comp. Biochem. Physiol.* **14**, 437.
 Ballard, F. J. & Hanson, R. W. (1967a). *Biochem. J.* **102**, 952.
 Ballard, F. J. & Hanson, R. W. (1967b). *J. Lipid Res.* **8**, 73.
 Balmain, J. H., Folley, S. J. & Glascock, R. F. (1953). *Biochem. J.* **52**, 301.
 Balmain, J. H., Folley, S. J. & Glascock, R. F. (1954). *Biochem. J.* **56**, 234.
 Barker, J. M. (1961). *Quart. J. exp. Physiol.* **46**, 54.
 Beinert, H., Green, D. E., Hele, P., Hift, H., Korff, R. W. von & Ramakrishnan, C. V. (1951). *J. biol. Chem.* **193**, 703.
 Buhler, D. R. (1962). *Analyt. Biochem.* **4**, 413.
 Dole, V. I. (1965). In *Handbook of Physiology: Adipose Tissue*, section 5, p. 13. Ed. by Renold, A. E. & Cahill, G. F., jun. Baltimore: Williams and Wilkins Co.
 Flatt, J. P. & Ball, E. G. (1964). *J. biol. Chem.* **239**, 675.
 Hanson, R. W. (1965). *Arch. Biochem. Biophys.* **109**, 98.
 Hardwick, D. C. (1965). *Biochem. J.* **95**, 46p.
 Hardwick, D. C. (1966). *Biochem. J.* **99**, 228.
 Henning, H. V., Stumpf, B., Ohly, B. & Seubert, W. (1966). *Biochem. Z.* **344**, 274.
 Holdsworth, E. S., Neville, E., Nader, C., Jarrett, I. G. & Filsell, O. H. (1964). *Biochim. biophys. Acta*, **86**, 240.
 Horecker, B. L. & Smyrniotis, P. Z. (1955). In *Methods in Enzymology*, vol. 1, p. 323. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
 Khachadurian, A. H., Adrouni, B. & Yacoubian, H. (1966). *J. Lipid Res.* **7**, 427.
 Kleiber, M., Smith, A. H., Black, A. L., Brown, M. A. & Tolbert, B. M. (1952). *J. biol. Chem.* **197**, 371.
 Kornberg, A. & Horecker, B. L. (1955). In *Methods in Enzymology*, vol. 1, p. 323. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
 Kronfeld, D. S. (1958). *Cornell Vet.* **48**, 394.
 Kronfeld, D. S., Mayer, G. P., Robertson, J. M. & Raggi, F. (1963). *J. Dairy Sci.* **46**, 559.
 Langdon, R. G. (1957). *J. biol. Chem.* **226**, 615.
 Leveille, G. A. & Hanson, R. W. (1966). *J. Lipid Res.* **7**, 46.
 McClymont, G. L. & Setchell, B. P. (1956). *Aust. J. biol. Sci.* **9**, 184.

- Ochoa, S. (1955a). In *Methods in Enzymology*, vol. 1, p. 685. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Ochoa, S. (1955b). In *Methods in Enzymology*, vol. 1, p. 739. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Roe, W. E., Bergman, E. N. & Kon, K. (1966). *Amer. J. vet. Res.* **27**, 729.
- Spencer, A. F. & Lowenstein, J. M. (1962). *J. biol. Chem.* **237**, 3640.
- Srere, P. A. (1959). *J. biol. Chem.* **234**, 2544.
- Srere, P. A. (1962). In *Methods in Enzymology*, vol. 5, p. 641. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1959). *Manometric Techniques*, p. 149. Minneapolis: Burgess Publishing Co.
- Utter, M. F. & Keech, D. B. (1960). *J. biol. Chem.* **235**, 17.
- Wakil, S. J., Porter, J. W. & Gibson, D. M. (1957). *Biochim. biophys. Acta*, **24**, 453.
- Winegrad (1965). In *Handbook of Physiology: Adipose Tissue*, section 5, p. 319. Ed. by Renold, A. E. & Cahill, G. F., jun. Baltimore: Williams and Wilkins Co.
- Wise, E. M., jun. & Ball, E. G. (1964). *Proc. nat. Acad. Sci., Wash.*, **52**, 1253.
- Young, J. W., Shrager, E. & Lardy, H. A. (1964). *Biochemistry*, **3**, 1687.