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Cholesterol and fatty acid synthesis in
ruminating and nonruminating goats

by

George Uldis Liepa

A Dissertation Submitted to the
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INTRODUCTION

A thorough understanding of lipid metabolism is of vital importance to man for several reasons. A primary reason is that dietary lipids have been associated with the development of atherosclerosis. Much work has implicated cholesterol and saturated fatty acids (Lambert et al., 1958; Krogh et al., 1961; Fetcher et al., 1967; McGill, 1968); however, many recent experiments reviewed by Jacobson (1974) indicate that numerous exceptions exist. Apparently, details concerning which and what amounts of dietary lipids accentuate atherosclerotic symptoms are still poorly understood.

Even though the dietary lipid relationship to atherosclerosis is unclear, dietary patterns of people in the United States reflect a decreased consumption of cholesterol and saturated fats on one hand and an increased consumption of polyunsaturated fats on the other. Because beef fat contains about 60% saturated fatty acids (Jacobson, 1974), many Americans feel its consumption is unhealthy. Approximately 2.5 billion pounds of excess fat are trimmed every year from beef carcasses (Bauman, 1974). Because much of this fat is discarded by Americans, we are making inefficient use of feed energy, and it would be to the world's advantage to decrease the flow of excess dietary energy to fat deposition in meat animals.

Man's physiological need for lipids also has led to increased interest in research on lipid metabolism. In addition to acting as a readily available source of energy, the physical characteristics of deposited lipids also allow them to function as insulators of delicate

body organs. Complex lipids are essential components of nervous tissue, cell membranes and membranes of subcellular particles such as mitochondria, microsomes and nuclei. Cholesterol helps provide membrane stability, and its presence is vital for controlling membrane permeability of different solutes (Papahadjopoulos, 1974). Phospholipids are an important constituent of cellular membranes also; 90% of the lipids in the mitochondrial membrane are phospholipids. Lipids are synthesized by several organs, excreted into body fluids and transported to other organs where they perform various functions. Sex hormones and adrenal hormones aid in body regulation, bile acids assist in lipid absorption in the small intestine and the fat-soluble vitamins (A, D, E and K) are necessary components of an animal's body.

Because atherosclerosis has become not only a physical but also an economic burden on Americans, it is imperative that we attempt to alleviate the problem. It is hoped that by increasing our understanding of in situ cholesterol and fatty acid synthesis, we can learn how to safely regulate the development of atherosclerosis and to ameliorate the problem. Because there is a significant relationship between blood cholesterol and atherosclerosis and because most blood cholesterol is synthesized within the animal itself, it is important to study the interaction between blood cholesterol and the de novo synthesis of cholesterol. Specifically, the objective of the experiments described in this dissertation was to measure in vitro rates of cholesterol and fatty acid synthesis from glucose and acetate in several tissues from young nonruminating and ruminating goats. The goat already has been demon-

strated to be an excellent animal model from which to study control of blood cholesterol and atherosclerosis in humans.

REVIEW OF LITERATURE

In this literature review, cholesterol and fatty acid synthesis and the body's mechanism for regulation of synthesis of these two lipids are discussed. After describing the metabolic pathways of cholesterol and fatty acid synthesis, the methods by which ruminants and nonruminants degrade dietary constituents to produce lipid precursors are discussed. Because end products of digestion in ruminants and nonruminants vary considerably, the two animal categories are independently reviewed in terms of how their major tissues (adipose, liver, brain and small intestine) synthesize cholesterol and fatty acids from dietary constituents. These topics have been reviewed in a number of excellent articles (Bauman, 1974; Dietschy and Wilson, 1970; Dhopeswarkar and Mead, 1972; Davison and Dobbing, 1968; Swann et al., 1975).

Cholesterol Balance in the Body

The two major origins of cholesterol within animals are diet and in situ synthesis. Figure 1 illustrates the major components of cholesterol balance in a normal human adult. In man, in situ synthesis accounts for approximately 66% of the cholesterol in the body pool; therefore, about 34% of it normally is derived from the diet. As blood (some dietary) cholesterol circulates through the liver, the cholesterol side chain may be oxidized, producing bile acids that are secreted into the small intestine by way of the bile. These bile acids, along with diet-, bile- and intestine-derived neutral sterols (primarily cholesterol), are then either absorbed from the intestine or excreted with the

Figure 1. Approximate cholesterol balance in the normal human adult

feces. Small amounts of sterol are also lost via skin, urine and the reproductive system. The most important concept illustrated in Figure 1 is that blood and tissue concentrations of cholesterol are determined primarily by the balance achieved between dietary input and body synthesis of cholesterol on the one hand and excretion of neutral sterols and cholesterol-derived bile acids on the other hand. This concept of cholesterol balance applies to other animals as well as humans (Naber, 1975).

Mechanism of Cholesterol and Fatty Acid Synthesis and Its Regulation by Feedback Inhibition

During the process of oxidation to CO_2 , proteins, carbohydrates and triglycerides are catabolized to the common intermediate, acetyl CoA. Acetyl CoA can then either be oxidized to CO_2 for ATP production via the citric acid cycle plus the electron transport system or be used as a precursor for larger molecules such as cholesterol and long chain fatty acids. Fatty acids, in turn, are incorporated into triglycerides and deposited as body fat. Figures 2 and 3 show the biochemical pathways for cholesterol synthesis from acetyl CoA and for fatty acid synthesis from glucose and acetate, respectively. When consumption of any of the three major dietary energy sources (protein, carbohydrate or lipid) are in excess of total body energy needs, then intermediates such as acetyl CoA increase in quantity and become increasingly available for fatty acid synthesis.

One of the principal means for avoiding excessive cholesterol synthesis within a particular tissue is the negative feedback system (Swann et al., 1975). It has been demonstrated by many researchers that

Figure 2. Biosynthetic pathway for cholesterol. The rate-limiting step is the conversion of 3-hydroxy-3-methyl glutaryl CoA to mevalonic acid. Enzymes catalyzing these reactions have been designated as follows:

1. Acetoacetyl CoA thiolase (EC 2.3.1.9)
2. 3-Hydroxy-3-methyl glutaryl CoA synthase (EC 4.1.3.5)
3. 3-Hydroxy-3-methyl glutaryl CoA reductase (EC 1.1.1.34)
4. Mevalonic kinase (EC 2.7.1.36)
5. Phosphomevalonic kinase (EC 2.7.4.2)
6. Pyrophosphomevalonate decarboxylase (EC 4.1.1.33)
7. Isopentenyl pyrophosphate isomerase (EC 5.3.3.2)

Figure 2. (Continued)

Enzymes:

8. Geranyl pyrophosphate synthetase (EC 2.5.1.1)
9. Farnesyl pyrophosphate synthetase (EC 2.5.1.1)
10. Presqualene synthetase

Figure 2. (Continued) Biosynthetic pathway of cholesterol.
Enzymes:

11. Squalene synthetase
12. Squalene epoxidase (EC 1.14.99.7)
13. Squalene oxide cyclase (EC 5.4.99.7)

Figure 3. Pathway for fatty acid biosynthesis from glucose and acetate. The circular figure represents a mitochondrion. Reactions occurring outside mitochondrion are occurring in the cytosol of the cell.

1. Acetate thiokinase (EC 6.2.1.1)
2. Pyruvate carboxylase (EC 6.4.1.1)
3. Pyruvate dehydrogenase (EC 1.2.4.1)
4. Citrate synthetase (EC 4.1.3.7)
5. Citrate cleavage enzyme (EC 4.1.3.6)
6. Acetyl CoA carboxylase (EC 6.4.1.2)
7. Fatty acid synthetase
8. Malate dehydrogenase (EC 1.1.1.37)
9. NADP-malate dehydrogenase (EC 1.1.1.40)

liver is the primary organ involved in cholesterologenesis in numerous species; consequently, effects of exogenous cholesterol on cholesterol synthesis has been observed mostly on this one tissue. A cholesterol-sensitive feedback system has been considered unique to the liver (Dietschy and Siperstein, 1967). This conclusion, however, was based upon a limited number of studies carried out with rats (Frantz et al., 1954), squirrel monkeys (Dietschy and Wilson, 1968) and dogs (Gould et al., 1953). More recently, Swann et al. (1975) found that in guinea pigs, the lung and small intestine were the most active sites of cholesterologenesis per unit weight of tissue and, more surprisingly, all tissues studied (lung, ileum, brain and liver) possessed an active, cholesterol-sensitive negative feedback system.

In liver of all species, a sensitive system of negative feedback control exists (Tomkins et al., 1953; Gould et al., 1953; Frantz et al., 1954; Siperstein, 1970). By this system, cholesterol production is impaired through the inhibition of mevalonic acid synthesis by cholesterol (Siperstein and Fagan, 1966; Linn, 1967). Specifically, cholesterol acts by inhibiting the synthesis of 3-hydroxy-3-methyl glutaryl CoA (HMG-CoA) reductase (Stryer, 1975). The inhibition is mediated by serum lipoproteins that bind to the plasma membrane of liver.

A number of studies also have been done on inhibition of lipogenesis (fatty acid synthesis) by dietary fatty acids. A high-fat diet fed to rats depresses lipogenesis in liver and adipose tissue (Masoro, 1962; Diller and Harvey, 1964; Leveille, 1967a,b). This effect also has been shown in adipose tissue of the pig (Allee et al., 1971a,b; Allee et al., 1971c) and in hepatic tissue of the chicken (Yeh and Leveille, 1969;

Yeh et al., 1970).

Experiments have been performed to determine if some fatty acids are more efficient feedback inhibitors than others. In the liver of mice, inhibition of lipogenesis is most closely related to linoleate content of the dietary fat (Smith et al., 1969; Smith and Abraham, 1970). Dietary corn oil, which contains a relatively high concentration of linoleate, suppresses fatty acid synthesis in pig adipose tissue (O'Hea and Leveille, 1969a). Other studies indicate that the inhibition of fatty acid synthesis by dietary fat is independent of its fatty acid composition. For example, Allee et al. (1972) reported that the addition of 10% dietary fat as corn oil, lard, tallow or coconut oil resulted in similar and marked depressions in conversion of glucose to fatty acids. Activities of both malic enzyme and citrate cleavage enzyme also were depressed. Therefore, dietary fat of any form, saturated or unsaturated, seems to suppress fatty acid synthesis in pig adipose tissue.

It is now thought that long-chain, free fatty acids are efficient inhibitors of lipogenesis in all species. They may limit fatty acid synthesis by increasing the concentration of acetyl CoA and, consequently, competing for coenzyme A (Masoro, 1962). Bortz and Lynen (1963) found that purified acetyl CoA carboxylase was inhibited by addition of long-chain fatty acyl CoA derivatives. This inhibition seems due to competition with acetyl CoA for the active site in the enzyme. Therefore, high concentrations of dietary fat decrease the rate of lipogenesis by way of its inhibition of acetyl CoA carboxylase.

Intracellular Site of Cholesterol and Fatty Acid Synthesis

Lipogenic cells contain a complex system of membranes and vesicles that permeate the major volume of extranuclear and extramitochondrial space. These intracellular membranes, also called the endoplasmic reticulum (ER), consist of both rough and smooth sections, with the smooth section containing the enzymes responsible for triglyceride synthesis from fatty acids and α -glycerophosphate. Enzymes for phospholipid synthesis, as well as glucose-6-phosphate phosphatase and several NADPH-linked oxidases and hydroxylases, are associated with the ER. Substrates for these enzymes are present in the cytosol and, in the case of triglyceride synthesis, the product is accumulated as droplets within the reticulum vesicles.

Mitochondria also are essential for lipid synthesis because they contain enzymes that function in the citrate cleavage pathway included in Figure 3 (Ballard and Hanson, 1967). The plasma and mitochondrial membranes are impermeable to acetyl CoA, the "building block" of fatty acids; fatty acids and pyruvate, however, can readily enter cells by diffusion. Fatty acids transverse the mitochondrial membrane as fatty acyl-carnitine, and pyruvate diffuses across as the free anion. Mitochondrial acetyl CoA, which is a product of fatty acid and pyruvate oxidation, however, can condense with oxaloacetate to produce citrate. Citrate, under conditions of high oxidizable substrate supply, accumulates and then diffuses out of mitochondria into the cytosol. After entering the cytosol from mitochondria, citrate may be cleaved by citrate cleavage enzyme (ATP-citrate lyase) to form oxaloacetate and acetyl CoA.

Thus, citrate cleavage enzyme provides cytosolic acetyl CoA for lipogenesis and, as expected, is markedly greater in concentration in cells that synthesize triglycerides. An important lipogenic enzyme in cytosol is malic enzyme. Oxaloacetate formed from citrate is reduced by NADH to form malate. The malate, then, is oxidatively decarboxylated by malic enzyme to form pyruvate that enters the mitochondrion. This latter reaction also generates NADPH which serves as a reductant in cholesterol and fatty acid synthesis. The net result of the citrate cleavage pathway is that cytosolic pyruvate is converted to cytosolic acetyl CoA for the lipid synthesis that occurs within the aparticulate portion of cytoplasm.

Smooth ER occurs extensively also in steroid-secreting cells. Enzymes active in steroid synthesis are abundant on smooth ER in these cells. Although the precise cellular location of all enzymes involved in cholesterol synthesis have not been determined, the most important regulatory enzyme, HMG-CoA reductase, is associated with the ER. The other ER-associated enzymes responsible for cholesterol synthesis are those that catalyze the reactions of squalene conversion to cholesterol. This rate-limiting enzyme of cholesterol synthesis from acetyl CoA is responsible for catalyzing the reduction of HMG-CoA (3-hydroxy-3-methyl glutaryl CoA) to form mevalonate. Recent evidence reviewed by Scallen et al. (1974) indicates that two sterol carrier proteins bind the intermediates of the squalene-to-cholesterol metabolic pathway. Although incompletely understood, sterol carrier protein I functions in the first part of the squalene-to-cholesterol pathway and sterol carrier protein II functions in the latter portion.

End Products of Digestion in Nonruminating and Ruminating Animals

Most carnivorous and omnivorous animals have one simple stomach which actively secretes pepsinogen and hydrochloric acid (Prosser and Brown, 1965). The principal function of the stomach is restricted to hydrolysis of dietary proteins. Ruminants, a type of herbivore, differ from the other two types of animals mentioned in that they can utilize cellulose. The rumen, a unique compartment in the ruminant's stomach, accomplishes this by maintaining a symbiotic relationship with cellulose-hydrolyzing microbial organisms. The cecum functions similarly in some types of animals.

In goats and cattle, one can approximate, by dietary manipulations, either the nonruminating or ruminating condition. By maintaining young goats or cattle on milk diets, digestion is similar to that in monogastric animals. Dietary carbohydrates contained in liquid feeds do not enter the undeveloped rumen because the esophageal groove closes during sucking; thus, soluble and water-suspended nutrients are shunted from the esophagus directly to the omasum. When this physiological situation exists, digestible carbohydrates are hydrolyzed within the small intestine, and the resulting hexoses are absorbed into the portal blood system. In animals with a functional rumen, solid feeds pass into the rumen and are converted primarily to the volatile fatty acids, acetate, propionate, butyrate and valerate (VFA), and microbial constituents. The VFA, then, can be directly absorbed into the portal blood system. The microbial constituents are hydrolyzed to absorbable products (e.g., amino acids and saturated fatty acids) in primarily the small intestines (Kiddle, et al., 1951). The VFA are the primary energy source for most of the animal tissues, whereas in

monogastric animals and nonruminating ruminants, glucose is usually the primary energy source.

Cholesterol and Fatty Acid Synthesis in Nonruminants

Intestinal lipid synthesis

Every mammalian tissue tested to date has been shown capable of some cholesterogenesis; the rates, however, do vary for different tissues (Srere et al., 1950; Dietschy and Siperstein, 1967; Dietschy and Wilson, 1968). In rats (Dietschy and Siperstein, 1967) and monkeys (Dietschy and Wilson, 1968), liver and small intestine have the highest rates of cholesterol synthesis. It is physiologically and clinically important to determine which tissues contribute significantly to the circulating cholesterol pool.

Before 1955, the liver was considered to be the only tissue that produced significant amounts of cholesterol for the blood serum pool (Hotto and Chaikoff, 1955). However, now it has been established that other tissues such as the intestinal mucosa also synthesize significant amounts of cholesterol which may enter the blood cholesterol pool. In humans fed a high cholesterol diet, 60 to 80% of their serum cholesterol arose from endogenous synthesis (Taylor et al., 1960; Wilson and Lindsey, 1965). Keeping in mind that cholesterol synthesis in livers of humans is inhibited by dietary cholesterol and that in the small intestine is not, (Bhattathiry and Siperstein, 1963), it seems probable that the intestine may, in cases such as high cholesterol intakes, exceed the liver in quantitative importance as a source of blood plasma cholesterol.

When comparing rates of cholesterol synthesis in different regions of the small intestine, it was found that the rat and the squirrel monkey fed no dietary cholesterol show similar patterns (Dietschy and Wilson, 1968; Dietschy and Siperstein, 1965; Dietschy, 1968). Maximal cholesterogenesis from acetate was observed in the terminal ileum, and these rates were unaffected by dietary cholesterol. Incorporation of acetate into cholesterol was much lower at the proximal end of the small intestine. The rat ileum synthesized cholesterol at a rate which is comparable to that in liver, whereas the proximal intestine synthesized cholesterol at one-twelfth of the hepatic rate (Dietschy and Wilson, 1968; Dietschy and Siperstein, 1965; Dietschy, 1968).

A similar regional pattern of cholesterol synthesis is noted in the small intestine of humans consuming about 98 to 280 mg of cholesterol daily (Dietschy and Gamel, 1971). Synthesis (tissue-weight basis) in proximal small intestine, however, is nearly equal to that in liver, and the rate in the ileum is four-fold greater than that in the liver. Long-chain fatty acids are synthesized at approximately equal rates throughout the small intestine, with only a slight increase in synthesis when moving from the proximal to the distal portion. Low rates of cholesterol and fatty acid synthesis were observed in the stomach mucosa and rectum.

O'Hea and Leveille (1968) measured cholesterol and fatty acid synthesis in different tissues of the pig and found that liver did not convert glucose to nonsaponifiable lipid (primarily cholesterol); however, when acetate was used as a substrate, liver synthesized significant amounts of nonsaponifiable lipids. More recent work from the same

laboratory (Romsos et al., 1971) showed that when pigs were maintained on a low-cholesterol diet and when acetate was used as precursor for cholesterol, liver was the primary site of cholesterolgenesis (67% of total); only 4% of the newly synthesized cholesterol was contributed by the small intestine. Cholesterol synthesis in the small intestine was greater in each of the upper three quarters of the small intestine than in the lower one.

Hepatic cholesterol and fatty acid synthesis

When attempting to determine the chief synthetic organs of cholesterol and fatty acid synthesis and to determine the relative importance of each organ, lipogenesis in the liver must be considered. It is well established that liver possesses the ability to synthesize triglycerides, phospholipids and fatty acids and, to a lesser extent, proteins (Guyton, 1971). This view has been modified because it has been found that livers from cattle, pigs and sheep showed negligible amounts of fatty acid synthesis (Hanson and Ballard, 1967, Mayfield et al., 1966; O'Hea and Leveille, 1969a).

In most animal species, liver is the main organ responsible for synthesis of body cholesterol. Specifically, liver of rats and monkeys is the primary site of cholesterol production; moreover, liver of monkeys synthesized about five times as much cholesterol per liver weight as that of rats (Dietschy and Wilson, 1970). In rats, liver produced twice as much cholesterol per tissue weight as did the small intestine (ileum); in the monkey, six times more hepatic cholesterol than ileal cholesterol is produced. Hepatic cholesterol synthesis in the monkey is equivalent to

82% of the total cholesterologenesis in the whole body. In humans, however, cholesterol is produced at almost equal rates per tissue weight in liver and small intestine (Taylor et al., 1955). Swann et al. (1975) found that liver of the guinea pig synthesizes cholesterol at a low rate, averaging only 6% of that in its ileum and only 4% of that in rat liver.

In many common animal species, liver is a minor contributor of body fatty acids. In the pig, organic acids such as acetic, propionic and lactic acid are produced in the lower digestive tract, appear in blood, and are removed by the liver (Friend et al., 1964); it is conceivable that acetate may serve as a precursor for hepatic fatty acid synthesis. In vivo and in vitro studies by O'Hea and Leveille (1969a) indicate that the fatty acid synthesis in liver accounts for about 25% of all newly synthesized fatty acids in the pig. Glucose was used poorly as a precursor of fatty acids in the liver. More than 99% of the de novo synthesis of fatty acids from glucose occurred in the adipose tissue. In contrast, when acetate was used as a precursor, synthesis was high in liver.

In chickens, hepatic tissue is the primary producer of fatty acids (Goodridge, 1968a; O'Hea and Leveille, 1969b); adipose tissue makes only a small contribution to total body fatty acid synthesis (Yeh and Leveille, 1973). In vivo studies with acetate as precursor indicate that chicken liver is four times more active than rat liver in regard to both fatty acid and cholesterol production (McIndoe, 1959; Heald and Badman, 1963). When a chicken starts producing eggs, the lipogenic activity of liver increases. The amount of lipid synthesized by the typical layer per year

is equal to the animal's body weight. Marked hyperlipemia is produced when the hepatic lipid is transported to the ovaries for secretion into eggs. With the hepatic tissue acting as a primary site for lipid synthesis, adipose tissue of birds then serves mainly in triglyceride reesterification and lipid storage (Leveille et al., 1975).

In humans, the relative contribution of liver and adipose tissue to total fatty acid synthesis is similar to that in chickens (Shrago et al., 1971; Patel et al., 1975). Human adipose is unable to incorporate glucose carbon into long-chain fatty acids (Patel et al., 1975). Under in vitro conditions, utilization of acetate by human adipose tissue for fatty acid synthesis is substantial; however, under normal physiological conditions, little acetate is available for lipogenesis. Apparently, the liver, not the adipose tissue, plays the major role in de novo fatty acid synthesis from dietary constituents in humans.

Pushpendran and Eapen (1973a) monitored in vivo fatty acid synthesis in mice and reported on variations in synthesis during postnatal development. In liver, rate of incorporation of glucose into fatty acid increased after weaning of mice. In rats, lipogenesis decreased immediately after birth, increased after weaning and then declined again during subsequent development (Ballard and Hanson, 1967; Taylor et al., 1967; Carroll, 1964). These fluctuations are related directly to dietary changes, especially the fat concentration in the diet. A recent in vivo study of fatty acid synthesis in the rabbit indicates that both liver and adipose tissue are the major sites of synthesis when either glucose or acetate is used as a precursor (Leung and Bauman, 1975). Relative

contribution of liver and adipose tissue to total fatty acid synthesis in the rabbit is dependent on age. In the young rabbit (2 to 3 kg body weight), over 50% of endogenously produced fatty acids were synthesized in liver; however, mature rabbits had an increased amount of body fat which accounted for 63 to 80% of the total body fatty acid synthesis.

Another factor which affects both lipid content in tissues and incorporation of acetate into lipids is the animal's gender (Perry and Bowen, 1958). For example, in mice, hepatic lipid content is higher in females than in males. Castration of males enhances hepatic lipogenesis whereas testosterone administration inhibits it.

Cholesterol and fatty acid synthesis by adipose tissue

Adipose tissues of different mammalian species (Farkar et al., 1973; Ho and Taylor, 1968), including that of man (Crouse et al., 1972), contain cholesterol that is in dynamic equilibrium with plasma cholesterol (Chohanian and Hollander, 1962; Samuel et al., 1972). Although the concentration of cholesterol in adipose tissue is quite small compared to that in liver, the storage fat forms one of the largest body cholesterol pools (Wilson, 1970); therefore, this tissue must be carefully studied in terms of total body or plasma cholesterol synthesis and turnover. Adipose tissue also can be very active in fatty acid synthesis with rates varying greatly from species to species.

Recently, Konvanen et al. (1975) conducted in vitro studies on cholesterogenesis by rat adipose tissue in which three cholesterol precursors (mevalonate, glucose, and acetate) were tested. Mevalonate was incorporated most readily, followed by glucose and then acetate. Rate

of cholesterol synthesis from any of the three substrates increased as fat cell size increased. When glucose was the precursor, insulin caused a three-fold increase in cholesterogenesis. In contrast to that in liver, the rate of cholesterol synthesis by adipose tissue was low. Adipose tissue synthesizes cholesterol at a rate that is only 2% of that observed in liver. In squirrel monkeys, the rates of cholesterol synthesis in both liver and adipose tissue were higher than that observed in the corresponding tissues of the rat; the relative percentages of synthesis by each tissue were similar to those for the rat (Dietschy and Wilson, 1968).

O'Hea and Leveille (1968) observed that the rate of cholesterogenesis in adipose tissue of pigs was low compared to its capacity of fatty acid synthesis. Although the rates of cholesterol synthesis are low in adipose tissue of nonruminants, the physiological importance of cholesterol synthesis in adipose tissue is unclear. Some work has suggested that cholesterogenesis in adipose tissue seems a significant factor in control of serum cholesterol concentrations.

In contrast to other peripheral tissues, cholesterogenesis in adipocytes of rats is markedly influenced by caloric intake (Konvanen et al., 1975). Fasting strongly suppresses cholesterogenesis, whereas refeeding after a fast caused an "overshoot" in cholesterol synthesis which was three times control rates. With the exception of the small intestine and adipose tissue, other extrahepatic tissues continue to synthesize cholesterol during fasting at a rate which is similar to or slightly lower than that occurring during the fed state.

Considerable work has been conducted on fatty acid synthesis in

adipose tissue and the variations of synthesis between species. In rats and mice, adipose tissue has a high rate of fatty acid synthesis compared to other tissues (Shafir and Wertheimer, 1965). In vivo studies on rats (Leveille, 1967b) and mice (Favarger, 1965) indicate that over 50% of total fatty acid synthesis occurs in adipose tissue. Insulin stimulated incorporation of glucose into fatty acids (Renold and Cahill, 1965). Adipose tissue seems a primary target for insulin and is the main synthesizing and storage organ of fatty acids in rats (Dole, 1965).

In vitro studies with pigs indicate that adipose tissue has a high lipogenic capacity (O'Hea and Leveille, 1968; O'Hea and Leveille, 1969a). Both glucose and acetate were readily utilized by adipose tissue for fatty acid synthesis; more glucose than acetate was incorporated into glyceride-glycerol. Differences in capacities of fatty acid synthesis in adipose tissue from different anatomical locations were reported; for example, subcutaneous adipose tissue possessed greater capacity than mesenteric adipose tissue.

Smith (1975) has shown that rabbit adipose tissue is capable of fatty acid synthesis from acetate and pyruvate. Adipose tissue showed a low capacity for fatty acid synthesis from glucose. Glucose was a poorer substrate for fatty acids than was pyruvate because glucose conversion to pyruvate seemed limited. Limiting activity of phosphofructokinase and pyruvate kinase was suggested to cause the difference in rates of glucose and pyruvate incorporation into fatty acids.

In the domestic chicken, little fatty acid synthesis occurs in adipose tissue (Leveille, et al., 1975; Goodridge, 1968a). The rate of

substrate incorporation per unit weight of rat adipose tissue exceeded that of chicken adipose tissue by a factor of 8 for acetate, 46 for pyruvate, and 274 for glucose (Leveille et al., 1975). Apparently, fatty acids are synthesized in the liver of chickens and are transported from liver via the blood as triglycerides in low-density lipoproteins to adipose tissue for storage or to other organs for other metabolic roles.

Human adipose tissue, like chicken adipose tissue, has a low capacity for fatty acid synthesis (Patel et al., 1975). Liver of humans plays the major role in the synthesis of endogenous fatty acids. Although adipose tissue can oxidize glucose to CO_2 , its ability to incorporate glucose into long-chain fatty acids is negligible. Acetate, in the presence of glucose and insulin, can be used for fatty acid synthesis; however, its physiological significance in humans under normal dietary conditions is questionable.

Cholesterol and fatty acid synthesis by neural tissue

Considerable research has been conducted concerning lipid synthesis in liver, small intestine and adipose tissue, but lipid synthesis in neural tissue has been less extensively studied. Because 78% of dry weight of bovine and human brain is lipid (McIlwain, 1966), obviously there has been a high rate of neural lipid synthesis and absorption occurring at some time in the animal's life. More effort needs to be devoted to study of the mechanism and control of lipid accumulation in neural tissue.

In any lipid synthesis study on brain tissue, two factors must always be considered: (1) the blood-brain barrier in the case of in vivo lipid

synthesis studies and (2) the developmental stage of the animal because of its impact on lipid synthesis rate. A study by Plotz et al. (1968) showed the importance of the blood-brain barrier in fetal brain development. Cholesterol synthesis was measured in human fetuses by injecting women during early pregnancy with radioactive acetate. Negligible amounts of isotope were incorporated into the cholesterol of the fetal brain; however, significant incorporation was found in the fetal liver and adrenal glands. In this same study, [^3H] cholesterol also was injected into the mother. Neither the [^3H] cholesterol nor the [$\text{U-}^{14}\text{C}$] cholesterol produced by the fetal liver and adrenal glands from the injected [^{14}C] acetate was transported into the fetal brain. However, when [$\text{U-}^{14}\text{C}$] glucose was injected into the mother, considerable isotope was found in cholesterol of the fetal brain and other fetal tissues. It was concluded that the blood-brain barrier in the fetus discriminates against blood acetate and cholesterol and, therefore, controls the movement of substances into and out of the central nervous system.

Jones et al. (1975) measured the passage of sodium acetate, glucose and mevalonic acid through the blood-brain barrier and the incorporation of each into cholesterol by brains of adult rats. In separate experiments, the three precursors were injected intracerebrally and intraperitoneally to determine the effect of the blood-brain barrier on precursor utilization by the brain. Glucose was the most readily used precursor; its incorporation into brain cholesterol was independent of the site of injection. This indicated that it readily traversed the blood-brain barrier. Acetate and mevalonate both were used more readily for chole-

terol synthesis when administered intracerebrally; however, when they were injected intraperitoneally, uptake and incorporation into cholesterol were still significant. A number of studies (Plotz et al., 1968; Davison and Dobbing, 1968; Portman, et al., 1972; Pushpendran and Eapen, 1973b) have been conducted which show the significant influence of age of the animal (developmental state) on the ability of its tissue to synthesize cholesterol. In these studies, it is generally shown that synthesis of lipid occurs most rapidly in the brain of immature animals. Until recently, it was thought that cholesterol synthesis occurred in neural tissue while the animal was very young, and only negligible synthesis occurred after puberty. In humans (Yakolev and LeCours, 1967), monkeys (Portman, et al., 1972) and rats (Serougne-Gautheron and Chevallier, 1973), myelination (and cholesterol synthesis) continues throughout life but only within certain anatomical regions of the brain. In a study done with adult rats by Chevallier et al. (1975), cholesterol synthesis was shown to occur even while there was no increase in brain weight. The constancy of brain weight was maintained by imposing dietary conditions of minimal economy on the rats.

In a similar study with rats, Ramsey et al. (1971) compared acetate and mevalonate incorporation into brain cholesterol. Intracerebrally injected mevalonic acid was converted to brain cholesterol much more efficiently than the intraperitoneally injected mevalonate. The intraperitoneally injected mevalonate was converted to cholesterol largely by the liver. Acetate, when injected intraperitoneally, traversed the blood-brain barrier more readily than mevalonate, and when injected at either

site, was converted more rapidly to brain cholesterol.

Patel and Tonkonow (1974) measured in vitro synthesis of cholesterol in brains from rats at birth through weaning. Maximum incorporation of acetate and glucose into brain cholesterol occurred at seven and twelve days of age, respectively. Pushpendran and Eapen (1973b) did an in vivo study on postnatal cholesterol synthesis in mouse brains. When acetate was used as a precursor, acetate incorporation into brain cholesterol occurred at an appreciable rate from birth to two and one-half months of age.

Fatty acid synthesis by brain has been studied extensively. Brady (1960) demonstrated that brain is as capable of synthesizing fatty acids as other organs such as liver. Although the major fatty acid synthesized by brain was palmitic acid, required enzymes are present for synthesis (either by de novo or chain elongation-desaturation processes) of all the necessary fatty acids except the essential fatty acids

Neural fatty acid synthesis in immature rats was studied by Patel and Tonkonow (1974). Maximum in vitro incorporation of acetate and glucose into fatty acids occurred at seven and twelve days, respectively; lipogenesis from either precursor was minimal by the twenty-fourth day. At 24 days of age, acetate was incorporated twice as fast as glucose. The rate-limiting step in de novo synthesis of long-chain fatty acid seemed to be acetyl CoA carboxylase.

Lipogenesis in brains of immature mice was measured by Pushpendran and Eapen (1973b). In vivo acetate incorporation into fatty acids occurred at a rapid rate for the duration of the experiments (two and one-half months). The highest rate of acetate incorporation was five days before

birth; the lowest was ten days after birth.

Dhopeshwarkar et al. (1971) measured incorporation of [$1-^{14}\text{C}$] acetate into brain lipids 15 seconds after injection of the substrate into the carotid artery, thus bypassing the liver. The specific radioactivity of total brain lipids was 10 times greater than that of the total liver lipids. Also, the following observations were noted: (1) considerable cholesterol synthesis from acetate had occurred, (2) palmitic acid had been synthesized from acetate, and (3) because all major polar lipid components were radioactive, incorporation of newly synthesized fatty acids into complex lipids had occurred.

Cholesterol and Fatty Acid Synthesis in Ruminants

In monogastric mammals given a high carbohydrate diet, lipids are formed primarily from glucose. Ruminants consume large amounts of carbohydrate in the form of cellulose, hemicellulose, and starch. These feed constituents are degraded into acetate, propionate and butyrate by the anaerobic microbes within the rumen (Eldsen and Phillipson, 1948). Acetate becomes the primary substrate available for de novo cholesterol and fatty acid synthesis. Rumen-derived propionate (and butyrate) is, however, largely removed from the portal blood during its passage through the liver (Leng and Annison, 1963), leaving acetate as the only volatile acid present in significant concentration in peripheral blood of ruminants (Reid, 1950). Therefore, little propionate and butyrate would be available to the various tissues for fatty acid synthesis.

Recently, some in vivo work has been conducted on cholesterol synthesis in mature goats (Thompson, 1975). In this study, acetate incorporation

into digitonin-precipitable sterols per tissue weight was reported. Adrenal gland showed the highest synthesis rates, then small intestine, liver, brain and adipose tissue in decreasing order. Acetate was incorporated into cholesterol by the small intestine at a rate that was 60 times that of liver. The small intestine mucosa was examined along its length at ten equally-spaced intervals; cholesterogenesis decreased from the orad to the caudad sections until the ileum. The ileum showed the most active rate of cholesterogenesis in the small intestine. When synthesis of digitonin-precipitable sterol was reported for whole organs on a per kilogram body weight basis, tissues ranked in the following decreasing order: skin, small intestine, adipose tissue, liver, adrenal gland and brain. Because goat skin has little cholesterol in its sterol fraction (Thompson, 1975), the small intestine and the adipose tissue seem the primary tissues of the mature goat involved in cholesterogenesis. Tepperman and Tepperman (1961) point out that a low rate of hepatic cholesterogenesis should be expected because the liver is involved primarily in gluconeogenesis, and gluconeogenesis and lipogenesis compete for carbon, reducing equivalents and ATP.

The majority of ruminant studies have involved two species, sheep and cattle. In vitro lipogenesis in adipose tissue of both bovine and ovine species has been studied by Ingle et al. (1972a). In nonlactating animals, the greatest rate of fatty acid synthesis was in adipose tissue; that in liver was only about 1% of that in adipose tissue. When measuring synthesis in the different sites of adipose tissue from young animals (lambs and calves), the internal fat depots were found most active; in mature ruminants (sheep and steers), however, the subcutaneous fat sites were found more active. In lactating animals, the mammary tissue was the

most active lipogenic tissue.

In an in vivo study of lipogenesis (Ingle et al., 1972b), liver, rumen, abomasum and small intestine together contributed only 8% of the total fatty acids synthesized de novo; the adipose tissue contributed 92%. Of seven different sites of adipose tissues tested, perirenal and omental adipose tissues had the greatest synthesis rates. Hood et al. (1972) observed that rates of lipogenesis per gram of subcutaneous adipose of steers was about 450-fold that observed in the same amount of liver. Rates per gram of intestinal mucosa were about 1% of that in subcutaneous adipose tissue. When glucose was used as a substrate, rates of synthesis in liver were about 10% of that in adipose tissue; however, rates in both tissues were markedly lower than when acetate was the substrate. Other studies agree with these results and show adipose tissue to be the most important site of lipogenesis in ruminants (Ballard et al., 1969; Al-Shathir, 1971).

Pothoven and Beitz (1973) studied lipogenesis in bovine adipose tissue and noted that on a tissue-weight basis, fatty acid synthesis in adipose tissue from several anatomical sites declined as steers grew older and larger. The rate of fatty acid synthesis in internal adipose sites of smaller animals was six-fold greater than that in large steers; in backfat, the synthesis was four-fold greater; and in intermuscular fat, it was three-fold greater. A number of other studies (Hood and Allen, 1975; Pothoven and Beitz, 1975; Ingle et al., 1972a), in general, agree with the above relationships of site of adipose tissue and rate of fatty acid synthesis.

The relative rates of incorporation of different substrates into fatty acids by ruminant tissues also have been studied. Hanson and Ballard (1967) compared rates of glucose and acetate incorporation into fatty acids in

sheep, cows and rats. Adipose tissue and liver were assayed. It was shown that in both tissues of the ruminants (sheep and cows), acetate was used more readily than glucose, whereas in the nonruminant (rat), glucose was incorporated more readily. Acetyl CoA synthetase was more active in ruminant tissues and thus allowed for formation of acetyl CoA from blood-derived acetate in the cytosol. The enzyme bypasses the need for citrate to transport acetyl CoA from the mitochondria. Also, activities of citrate cleavage enzyme and malic enzyme were low in ruminant tissues, causing glucose to be incorporated into fatty acids at a low rate. Non-ruminants have high citrate cleavage enzyme activity in lipogenic tissues and, therefore, the incorporation rate of glucose into fatty acids is high. High citrate cleavage pathway activity was expected because glucose is the chief precursor of fatty acids in nonruminants and citrate cleavage enzyme is essential for the production of cytosolic acetyl CoA. Hood et al. (1972) concur with Hanson and Ballard, because they found that adipocytes from cattle synthesized fatty acids more readily from acetate than from glucose and propionate.

Several compounds have been demonstrated to stimulate in vitro fatty acid synthesis in ruminant tissues. When adipose tissue from calves or lambs was incubated with acetate as the only carbon source, lipogenesis was only about 25% of that found when adipose tissue was incubated with acetate in the presence of glucose (Ingle, Bauman, and Garrigus, 1972b). Glucose addition did not stimulate acetate incorporation into fatty acids by liver. The stimulatory effects of glucose occur because glucose is a substrate for the dehydrogenases of the hexose monophosphate pathway which provides NADPH for fatty acid synthesis. Addition of insulin to the

incubation media for measuring fatty acid synthesis from acetate is slightly stimulatory.

PART I. CHOLESTEROL SYNTHESIS IN RUMINATING AND NONRUMINATING GOATS

MATERIALS AND METHODS

Experimental Animals

To study cholesterol synthesis, tissues from five hay- and grain-fed and five milk-fed Saanen goats at four months of age were assayed for in vitro cholesterol synthesis. One goat from each set of five twins was assigned to either the hay- and grain-fed or milk-fed group. The non-ruminating goats were fed goat milk at a rate equivalent to 15% body weight per day for the four-month feeding period. The other five goats were fed goat milk at the same rate for the first 30 days and then were weaned onto a hay and grain diet. Alfalfa hay was fed ad libitum and grain (Table 1) was fed at a rate equivalent to 2.5% of body weight per day. Diet of milk-fed goats also was supplemented with the vitamin-

Table 1. Composition of grain mixture

Component	% of Total ¹
Ground shelled corn	40
Rolled oats	27
Soybean meal (44% protein)	20
Molasses (cane)	10
Dicalcium phosphate	2
Sodium chloride	1
Vitamin-antibiotic mix ²	0.002
Trace minerals	Salt block ³

¹Percentage of air-dry feed.

²Calf starter (454 kg) contained 700 g of vitamin premix that consisted of: Vitamin A palmitate, 78 g; Vitamin D₂, 52g; and an antibiotic mix, 570 g (1 part chlortetracycline and 3 parts cracked corn).

³All goats were given free access to a salt block which contained (in %): NaCl, 97-98.5; CoCO₃, 0.011; ZnO, 0.005; MnO, 0.25; FeO, 0.25; CaO, 0.33; CaIO₄, 0.007; and MgO, 0.60.

antibiotic premix¹.

All goats were weighed weekly. Once every two weeks, jugular blood samples were collected into heparinized test tubes and stored at -20° for subsequent determination of cholesterol concentration (Technicon, 1965).

Tissue Collection

When four months of age, goats were killed by forced breathing of carbon dioxide and subsequent exsanguination. A sample of blood was collected, and the plasma was isolated and stored at -20°. Samples of brain, liver and perirenal adipose tissue and the whole small intestine were removed from each goat within 10 minutes of death and immediately placed in 39° normal saline. All remaining portions of organs were removed and weighed to obtain total organ weight. Cerebral brain samples were separated into gray and white fractions. The first 30.5 cm of proximal small intestine were removed and discarded, and the remainder of the small intestine was divided into 150-cm sections. A 60-cm portion of each 150-cm section of intestine was then removed and washed with normal saline. Washed intestine was then blotted dry and villi and crypts were removed with a razor blade (Dietschy and Siperstein, 1965). Weights obtained from these samples were then used for calculating the total weight of the small intestine. The most proximal, medial and distal sections of the small intestine each provided the mucosal samples used for incubation.

¹Goat milk (15 kg) contained 1029 g of vitamin premix which consisted of: Vitamin A palmitate, 9 g; Vitamin A palmitate/D₃ (300,000 I.U. vitamin A/g and 300,000 I.U. D₃/g), 1 g; Vitamin E (dl-alpha-tocopheryl acetate, 100,000 I.U./454 g), 720 g; chlortetracycline, 44 g; and MgO, 255 g.

Assay of Cholesterogenesis

Samples of each tissue (100 to 200 mg) were transferred to Erlenmeyer flasks (25 ml) containing 3.0 ml of Krebs and Henseleit Ringer-bicarbonate (Ca^{++} -free) buffer, pH 7.4 (Laser, 1961). Each flask also contained 30.0 μ moles of glucose and 0.3 units of bovine insulin. When glucose incorporation into cholesterol was assayed, 2.0 μ Ci of [$\text{U}-^{14}\text{C}$] glucose was added. When acetate incorporation was assayed, 75 μ moles sodium acetate and 2.0 μ Ci of [$2-^{14}\text{C}$] acetate were added. When incorporation of mevalonic acid and β -hydroxybutyric acids were assayed, the following concentrations of substrate were used: 30 μ moles of mevalonic acid plus 1 μ Ci of [$2-^{14}\text{C}$] mevalonic acid or 30 μ moles of β -hydroxybutyrate plus 1 μ Ci of [$3-^{14}\text{C}$] β -hydroxybutyrate. Zero time samples served as controls. The incubation conditions were such that incorporation into product was linear with respect to time of incubation and amounts of tissue assayed. Also, concentrations of glucose and acetate gave V_{max} conditions. V_{max} conditions for mevalonate and β -hydroxybutyrate were not established. Triplicate tissue samples were incubated for two hours according to the procedure outlined by Pothoven and Beitz (1973). Lipid was extracted from incubated tissues and washed by the method of Folch et al. (1957). After washing, the lipid extracts were evaporated and nonsaponifiable lipids (primarily cholesterol) were removed according to the procedure of Pothoven and Beitz (1973). Cholesterol residues obtained after evaporation of the cholesterol-containing extracts were dissolved in toluene containing PPO (0.3% and POPOP (0.017%) for scintillation counting. Carbon dioxide produced during incubation of tissues was collected and assayed for radioactivity according to the method described by Buhler (1962).

Statistical Analysis

Split plot analysis (Snedecor and Cochran, 1967) was used to analyze the data and P values less than 0.05 were considered to be significant.

RESULTS

Incorporation of [$1\text{-}^{14}\text{C}$] acetate and [$\text{U-}^{14}\text{C}$] glucose into non-saponified lipids was used to measure cholesterol synthesis in ruminating (R, hay- and grain-fed) and nonruminating (NR, milk-fed) goats. Throughout this paper, nonsaponifiable lipids will be referred to as cholesterol. Other work with the goat (Thompson, 1975) has demonstrated that skin is the only organ that synthesizes significant amounts of nonsaponified lipids which are not primarily cholesterol (digitonin-precipitable sterol). Rates of cholesterol synthesis were calculated on the basis of tissue weight and are reported as nmoles of substrate incorporated per two hours per gram tissue. Also, cholesterol synthesis rates were calculated on the whole organ (tissue) basis, and synthesis in each tissue was reported as a percentage of total body synthesis. Total body cholesterol synthesis will refer to the sum of synthesis in each tissue or organ measured. Unless specifically stated, the data of acetate incorporation under discussion will be that obtained from incubations when glucose also was present.

Rate of Tissue Cholesterogenesis

If cholesterol synthesis was expressed on a tissue-weight basis, adipose tissue was the primary cholesterogenic tissue when acetate was the precursor (Table 2). When glucose was used as precursor, rates of cholesterogenesis in adipose tissue and small intestine were similar; in NR goats, synthesis in brain also was similar to that in adipose tissue. With glucose as a precursor, rate of cholesterol synthesis was lowest in the liver; however, when acetate was precursor, brain and liver of NR goats had similar rates, but the rate in brain of R goats was lower than that in liver. Cholesterol synthesis from either substrate by R and NR goats was

Table 2. Utilization of acetate and glucose for cholesterol synthesis in ruminating and nonruminating goats

Tissue	Acetate and glucose		Glucose	
	R ¹	NR ²	R	NR
nmoles substrate incorp./ (2 hr x g tissue)				
Adipose tissue	341 ± 136 ^{3,ab}	227 ± 68 ^{ab}	11.2 ± 4.6 ^b	8.5 ± 3.2 ^b
Small intestine ⁴	62.2 ± 9.0 ^{arc}	31.8 ± 10.0 ^{ac}	13.2 ± 3.6 ^{rb}	5.4 ± 1.6 ^b
Proximal	43.8 ± 7.1 ^a	29.7 ± 9.1 ^a	8.8 ± 1.6 ^r	2.7 ± 1.0
Medial	67.8 ± 10.7 ^{ra}	17.1 ± 4.1	18.9 ± 6.0 ^r	3.6 ± 0.9
Distal	75.1 ± 9.3 ^{ra}	48.7 ± 16.7 ^a	11.9 ± 3.3	9.9 ± 2.8
Brain ⁴	6.6 ± 0.9 ^d	7.2 ± 1.6 ^d	4.2 ± 0.6 ^c	4.8 ± 0.7 ^b
Gray	2.6 ± 0.8 ^a	3.1 ± 1.2 ^a	0.9 ± 0.2	0.8 ± 0.1
White	10.6 ± 0.9	11.3 ± 2.0	7.5 ± 1.0	8.8 ± 1.3
Liver	13.2 ± 1.3 ^{rae}	3.5 ± 3.8	0.6 ± 0.3 ^d	0.3 ± 0.1 ^c

¹R refers to ruminating (hay- and grain-fed) goats.

²NR refers to nonruminating (milk-fed) goats.

³Mean ± SEM.

⁴Averages were calculated assuming equal weights in each tissue subdivision.

^rSignificant difference ($P < 0.05$) compared with NR goats (within tissue and substrate comparisons).

^aSignificant difference ($P < 0.05$) compared with glucose substrate (within tissue and diet comparison).

^{b,c,d,e}Values in the same column not sharing a common superscript letter are significantly different ($P < 0.05$). Comparisons were not made for individual sections of small intestine and brain.

similar; however, acetate was incorporated at a rate that was about 30 times greater than that of glucose in both groups of goats.

When acetate was used as a precursor, rate of cholesterol synthesis in the small intestine (as well as in each of the three intestinal sections) of both types of goats was lower than that in adipose tissue of the same goats. Rates in the small intestine were greater than those in brain and liver. Ruminating goats showed greater rates of intestinal cholesterogenesis than NR goats. Within the intestine, the distal section of the small intestine had the greatest synthetic capacity.

Cholesterogenesis from glucose in the small intestine of R and NR goats was similar. In addition, synthesis in the small intestine was similar to that in adipose tissue. Synthesis in the small intestine of R goats was higher than that observed in both brain and liver; in NR goats, synthesis in small intestine was similar to that in brain but higher than that in liver. As in adipose tissue, glucose incorporation into cholesterol was lower than that of acetate in both types of goats. In both R and NR goats, glucose incorporation was lower in the proximal section of the small intestine. In R goats, the highest rate was in the medial section, and in NR goats, the rates increased slightly when moving from the proximal to the distal section.

In brain, no difference was noted in rates of cholesterogenesis in R and NR goats. Also, the type of precursor seemed to have little effect on rate of cholesterogenesis. With few exceptions, cholesterogenesis in brain was less than that in adipose tissue and small intestine and greater than that in liver of R and NR goats. Brain gray or white matter of R and NR goats had similar incorporation rates when either glucose or acetate was used as precursor. In all goats, brain white matter incor-

porated glucose and acetate at rates that were four to eleven times greater than those in brain gray matter.

When acetate was used as precursor, livers of R goats synthesized cholesterol at three times the rate of that in livers of NR goats. In R goats, synthesis in liver was two-fold greater than that in brain and about one-fifth of that in small intestine. Rates of synthesis in brain and liver of NR goats were similar. When glucose was used as precursor, similar rates of cholesterol synthesis occurred in livers of R and NR goats. Rates in liver were less than 10% of that in brain.

To summarize the preceding section on cholesterol synthesis, there is a higher rate of cholesterogenesis in almost all tissues of R goats than in corresponding tissues from NR goats; in both types of goats, acetate is the preferred precursor for cholesterol synthesis.

Acetate incorporation into cholesterol also was examined without glucose being present in the incubation medium (Table 3). Rates of incorporation were determined for small intestine, adipose tissue and brain. Statistical analysis was not performed on these data. In all tissues, there was decreased acetate incorporation when glucose was excluded from the incubation media. The tissue with largest dependence on glucose addition was the adipose tissue (95% decrease in R goats and 79% decrease in NR goats when glucose was omitted). The medial section of the small intestine showed a decrease of 35% in R goats and 11% in NR goats. Gray matter in R goats had an 85% decrease in synthesis and that of NR goats, a 26% decrease; white matter decreased 41% and 52% in R and NR goats, respectively.

Table 3. Acetate incorporation (in absence of exogenous glucose) into cholesterol in ruminating and nonruminating goats

Tissue	Rate		Percentage ³	
	R ¹	NR ²	R	NR
	nmoles substrate incorp./ (2 hr x g tissue)		% ³	
Adipose tissue	18.5 ± 7.8 ⁴	47.3 ± 13.5	5.4	20.8
Small intestine (medial)	44.2 ± 9.6	15.2 ± 4.1	65.2	88.9
Brain				
Gray	0.4 ± 0.2	2.3 ± 1.3	15.4	74.2
White	6.3 ± 1.1	5.4 ± 1.2	59.4	47.8

¹R refers to ruminating (hay- and grain-fed) goats.

²NR refers to nonruminating (milk-fed) goats.

³Calculated as a percentage of the rate of acetate incorporation in the presence of glucose.

⁴Mean ± SEM.

Cholesterogenesis in Whole Organ

Adipose tissue made the greatest contribution to total cholesterol synthesis in both R and NR goats when acetate was used as a precursor (Table 4). When glucose was used as precursor, adipose tissue of NR goats contributed 60% of total body cholesterol synthesis; however, adipose tissue in R goats contributed only 29% of total body cholesterol synthesis.

Cholesterogenesis also occurred extensively in the small intestine. When acetate was used as a precursor, the small intestine of R goats

Table 4. Synthesis of cholesterol in whole organs of ruminating and nonruminating goats¹

Tissues	Acetate and glucose		Glucose	
	R ²	NR ³	R	NR
Adipose tissue	71.1 ± 5.8 ^{4,r}	90.0 ± 2.3	28.8 ± 13.0	60.2 ± 17.3
Small intestine ⁵	22.3 ± 2.7 ^r	6.5 ± 1.1	58.4 ± 11.6 ^r	19.5 ± 4.0
Brain ⁵	1.2 ± 0.2	1.8 ± .5	9.9 ± 2.0	18.7 ± 7.1
Liver	5.3 ± 3.5 ^r	1.0 ± .6	2.9 ± 1.5	1.5 ± 0.8

¹Data are expressed as percentage of total synthesis in all four tissues.

²Refers to ruminating (hay- and grain-fed) goats.

³NR refers to nonruminating (milk-fed) goats.

⁴Mean ± SEM.

⁵Average of data for three sections of intestine and two sections of brain.

^rSignificant difference (P < 0.05) compared with NR goats (within tissue and substrate comparisons).

contributed 22% of total body cholesterol synthesis and that of NR goats, 7%. With glucose as precursor, the proportion of total body cholesterol synthesis occurring in the small intestine increased to 59% and 20% in the R and NR goats, respectively.

The percentage of body cholesterol synthesis occurring in brain was 10% in R goats and 19% in NR goats when glucose was used as a precursor. With acetate, less than 2% of total body cholesterol synthesis occurred in brain. Incorporation of each substrate into cholesterol by brain of R and NR goats was similar. Acetate incorporation by brain of both types of goats was about 2% of that in the small intestine while glucose incorporation was about 30% of that in small intestine.

With the exception of acetate incorporation in liver of R goats, liver had the lowest amount of cholesterol synthesis, accounting for only 1 to 3% of the total body cholesterol synthesis. Liver of R goats produced a larger amount of cholesterol from acetate than did liver of NR goats.

Cholesterol synthesis from Mevalonate and β -hydroxybutyrate

Capacities of cholesterol synthesis from mevalonate and β -hydroxybutyrate in a section of small intestine and adipose tissue were assayed. When mevalonate was used as a precursor, cholesterol synthesis was higher in R goats. In adipose tissue, synthesis was 1.5 times greater in R goats than in NR goats (424 vs. 293 nmoles/[g x 2 hr]); in the distal small intestine,

it was four times greater in the R goat (247 vs. 72). β -Hydroxybutyrate was incorporated one and one-half times more readily by NR goats in both tissues (15.3 vs. 10.8 in adipose and 46.9 vs. 28.4 in small intestine).

When comparing rates of cholesterologenesis from mevalonate, β -hydroxybutyrate, acetate and glucose, adipose tissue used acetate and mevalonate at similar rates and 25 times more readily than either glucose or β -hydroxybutyrate. Cholesterologenesis from acetate or mevalonate occurred four times faster in adipose tissue than in small intestine. Mevalonate and acetate again had similar and highest rates of incorporation in the small intestine, β -hydroxybutyrate was incorporated at two-thirds of their rates, and glucose at one-seventh of their rates.

Glucose and Acetate Oxidation to CO_2

Of the four different tissues assayed, adipose tissue showed the highest rate of oxidation of acetate to CO_2 (Table 5). The rate of oxidation of glucose in adipose tissue was 8% of that for acetate in R goats and 22% of that for acetate in NR goats. Rates of oxidation of either substrate to CO_2 was similar in R and NR goats.

Oxidation of acetate to CO_2 in the small intestine was 30% of that in adipose tissue, whereas glucose oxidation to CO_2 in the same two tissues of R goats was similar and two-fold greater than that in adipose tissue of NR goats. With either type of goat, oxidation of glucose or acetate by the three sections of small intestine were similar.

There was no difference between rates of oxidation of acetate or glucose to CO_2 in brain of R and NR goats. Glucose, however, was oxidized at a rate about two times greater than that for acetate. Acetate oxidation to CO_2 in brain was 7 and 12% of the rate in adipose tissue of R and NR goats, respectively. In contrast, glucose oxidation in brain was 1.8 and

Table 5. Glucose and acetate oxidation to CO₂ by several tissues of ruminating and nonruminating goats

Tissue	Acetate and glucose		Glucose	
	R ¹	NR ²	R	NR
nmoles substrate incorp./ (2 hr x g tissue)				
Adipose tissue	10372 ± 6587 ^{3,ab}	7768 ± 1306 ^b	822 ± 9 ^b	1697 ± 578 ^b
Small intestine ⁴	3133 ± 511 ^{ac}	2154 ± 998 ^{ac}	718 ± 112 ^b	526 ± 171 ^c
Proximal	3816 ± 657 ^a	1887 ± 814 ^a	706 ± 122	438 ± 63
Medial	3108 ± 343 ^{ra}	2317 ± 1706	882 ± 131	620 ± 340
Distal	2467 ± 533 ^a	2258 ± 475 ^a	567 ± 85	522 ± 109
Brain ⁴	742 ± 322 ^{ad}	942 ± 321 ^{ac}	1446 ± 264 ^c	2195 ± 159 ^b
Gray	533 ± 122 ^a	918 ± 433 ^a	1750 ± 406	2775 ± 51
White	951 ± 522 ^a	965 ± 208 ^a	1143 ± 122	1616 ± 268
Liver	2078 ± 450 ^{ac}	2044 ± 1418 ^{ac}	310 ± 77 ^d	223 ± 4 ^d

¹R refers to ruminating (hay- and grain-fed) goats.

²NR refers to nonruminating (milk-fed) goats.

³Mean ± SEM.

⁴Averages were calculated assuming equal weights in each tissue subdivision.

^rSignificant difference (P < 0.05) compared with NR goats (within tissue and substrate comparison).

^aSignificant difference ($P < 0.05$) compared with glucose substrate (within tissue and diet comparison).

^{b,c,d}Values in the same column not sharing a common superscript letter are significantly different ($P < 0.05$). Comparisons were not made for individual sections of small intestine and brain.

1.3 times greater than the rate in adipose tissue of R and NR goats, respectively. Gray and white matter of brain had similar rates of oxidation of either precursor to CO_2 .

Oxidation of acetate to CO_2 by liver is three- to five-fold less than that in adipose tissue and similar to that in small intestine and brain. Glucose oxidation in liver, however, is two- to ten-fold less than that in small intestine and brain as well as adipose tissue.

Acetate is oxidized in liver at a rate seven and nine times that of glucose oxidation in R and NR goats, respectively.

In summarizing this section, acetate is oxidized to CO_2 most readily by adipose tissue and least readily by liver. Brain oxidizes glucose to CO_2 more readily than other tissues and liver does so least readily.

Body Weight Gain and Plasma Cholesterol

Goats were weighed periodically and showed an average weight gain of 0.51 kg per week. The final weights of the R and NR goats were similar (12.0 ± 0.1 and 12.2 ± 1.2 , respectively).

The cholesterol concentrations in blood plasma of the goats during the experimental period are presented in Figure 4. Cholesterol concentration in the R goats averaged 125 mg per 100 ml of plasma during the last three months of the experiment; however, the cholesterol concentration in the NR goats continued to increase during the four-month experiment, attaining a concentration of about 275 mg per 100 ml at the termination of the experiment.

Figure 4. Plasma cholesterol concentrations of ruminating and nonruminating goats

Values are expressed as mean + or - SEM.

NR refers to nonruminating (milk-fed) goats and R refers to ruminating (hay- and grain-fed) goats.

DISCUSSION

Before a proper understanding of the control of cholesterologenesis in any species can be attained, it is important to identify the main synthetic sites in the body and the relative importance of each. In the experiments reported here, in vitro techniques have been used to determine the importance of adipose tissue, small intestine, brain and liver of R and NR goats. The results presented agree with earlier work which demonstrated that adipose tissue of ruminants and swine seemed to synthesize cholesterol more readily than hepatic tissue (Hanson and Ballard, 1967; Huang and Kummerow, 1976). It also is shown that cholesterologenesis occurs more rapidly in adipose tissue than in either small intestine or brain.

The rate of cholesterologenesis also was found to vary significantly with different precursors. Hanson and Ballard (1967) showed that acetate was the preferred precursor over glucose in adipose tissue and liver of both sheep and cattle. Both tissues also showed low activities of citrate cleavage enzyme and high activities of acetyl CoA synthetase which correlated well with high rates of acetate incorporation and low rates of glucose incorporation. Goodwin (1968) documented the key role played by citrate cleavage enzyme in lipid synthesis, and it is now generally accepted that ruminants have a low capacity for cholesterol synthesis from glucose. Because my work shows that acetate is incorporated more readily than glucose in almost all tissues of NR as well as R goats, it seems that these animals can readily convert acetate to acetyl CoA and thus use acetate in preference to glucose as a cholesterol precursor. Based on others' results (Ballard and Hanson, 1967), acetate and glucose incorporation seemed positively correlated with the specific activities of acetyl CoA synthetase and citrate cleavage enzyme, respectively.

The present results indicate that adipose tissue was the primary cholesterogenic tissue in R and NR goats when acetate was used as a precursor. When glucose was used, adipose tissue and small intestine synthesized cholesterol at equal rates in R goats; in NR goats, adipose tissue, brain and small intestine synthesized cholesterol at similar rates. Earlier work (Hanson and Ballard, 1967) compared rates of cholesterol synthesis in adipose tissue and liver of ruminating (sheep and cattle) and nonruminating (rats) animals. Their results also showed that synthesis rates were consistently higher in the adipose tissue than in other tissues of ruminants. However, in rats, a higher rate of synthesis was found to occur in liver than in adipose tissues.

Because organic acids such as acetic, propionic and lactic acids are produced in the digestive tract of pigs (Friend et al., 1963a, Friend et al., 1963b) and appear in the blood stream (Barcroft et al., 1944; Friend et al., 1964), it is likely that mature swine can use them as precursors for cholesterol synthesis. Huang and Kummerow (1976) studied incorporation of acetate and glucose into cholesterol in adipose tissue, liver, heart and aorta of swine. When glucose and acetate were used as precursors, adipose tissue was the primary cholesterogenic tissue; lesser substrates were incorporated into cholesterol by liver, heart and aorta. Acetate was used for cholesterologenesis in preference over glucose.

Greater rates of cholesterol synthesis from acetate occurred in the small intestine of R goats than in NR goats. This was expected because the total mass of intestinal mucosa was greater in the R goats and because mucosa of roughage-fed animals undergoes greater sloughing off and therefore seems to have a greater regeneration rate (Prosser and Brown, 1965). However, the results of glucose incorporation do not support this reason-

ing because small intestine of R and NR goats showed similar rates of cholesterol synthesis from glucose. The distal portion of the small intestine showed the highest rate of cholesterol synthesis. In agreement with this finding, Huang and Kummerow (1976) observed that the highest rate of cholesterol synthesis in small intestine of swine was in the distal section when either glucose or acetate was used as precursor. The lowest rate of synthesis in the intestine was in the proximal section; this also was observed in goats. The Huang and Kummerow study and those of others involving cholesterogenesis in the small intestine of rat, monkey and man (Dietschy and Siperstein, 1967; Dietschy and Wilson, 1968; Dietschy and Wilson, 1970) show that the ileum demonstrates the highest rate of intestinal cholesterogenesis. However, data obtained from young swine (Romsos et al., 1971) contrast with this general statement in that the proximal section seemed to contribute the largest amount to intestinal cholesterogenesis.

In brain, the types of precursors and diets of goats had little effect on the rates of cholesterol synthesis. Brain white matter synthesized cholesterol at a higher rate than gray matter when either precursor was used. This was anticipated because the white matter of the brain contains a higher percentage of cholesterol than does the gray matter (McIlwain, 1966). Because the cholesterol content of brain of 4-month old goats probably has attained a maximum, the data of cholesterogenesis indicate that cholesterol of white matter undergoes a more rapid turnover than that in gray matter. This study is in agreement with other studies that show cholesterol synthesis still occurs in brain of mature animals (Pushpendran and Eapen, 1973b; Ramsey et al., 1971; Kabara, 1972; Chevallier et al., 1975). Furthermore, work with the goat showed that cholesterol synthesis

in brain occurs at a greater rate when compared to that in liver. Swann et al. (1975) also observed similar rates of cholesterologenesis from glucose or acetate in brain and liver of guinea pigs.

When either glucose or acetate was used as a precursor, there was a very low rate of cholesterol synthesis in goat liver. Glucose was incorporated at similar rates by liver of goats fed either diet; however, acetate incorporation occurred more readily in R goats. Low rates of cholesterol synthesis by hepatic tissues also has been shown in cattle and sheep (Hanson and Ballard, 1967), swine (Huang and Kummerow, 1976) and guinea pigs (Swann et al., 1975). Hanson and Ballard (1967) showed that cholesterol synthesis from glucose and acetate in liver was one-hundredth and one-half of the rates obtained for adipose tissue, respectively.

Table 6 shows the relative rates of acetate incorporation into cholesterol and into fatty acids by tissues from R and NR goats. Of the acetate incorporated into cholesterol and fatty acids in adipose tissue, 99% is incorporated into fatty acid and 1% into cholesterol. In the small intestine, acetate is incorporated into cholesterol and fatty acids at similar rates. In brain, 60 to 80% of lipid synthesis from acetate is accounted for by fatty acid synthesis. Glucose incorporation into cholesterol and fatty acids is shown in Table 7. With regard to glucose incorporation into cholesterol and fatty acids, 95% of glucose is used for fatty acid synthesis and only 5% for cholesterol synthesis. Small intestine and liver have approximately equal amounts of glucose being converted to cholesterol and fatty acids. Of the glucose used for cholesterol and fatty acid synthesis, brain gray matter converted 90% of the glucose to fatty acids, whereas the corresponding value for white matter was 50%. Huang

and Kummerow (1976) obtained data which was very similar to that presented. When either glucose or acetate are used as a precursor in adipose tissue, 99% of the substrate converted to cholesterol and fatty acids was used for fatty acid synthesis and the remainder for cholesterol synthesis. Similar to that in the goat, the corresponding value for glucose incorporation into fatty acids was 55%; for acetate, it was 75%.

Adipose tissue and small intestine were used to compare the incorporation rates of β -hydroxybutyrate, mevalonate, acetate and glucose. Glucose was incorporated at a rate similar to β -hydroxybutyrate in adipose tissue. Acetate and mevalonate incorporation was 25 times faster than glucose and β -hydroxybutyrate incorporation in adipose tissue. β -hydroxybutyrate is incorporated three times faster than glucose is in adipose tissue and acetate and mevalonate are incorporated slightly faster than β -hydroxybutyrate. Because mevalonate is an intermediate between acetate and cholesterol, it would be expected to have a high incorporation rate, and this has been shown to be the case in both brain and adipose tissue (Jones et al., 1974; Kovanen et al., 1975). In order for β -hydroxybutyrate to be incorporated into cholesterol, it must be oxidized to form acetyl CoA. No information is available in the literature with which to compare the β -hydroxybutyrate incorporation data of this experiment.

Acetate and glucose oxidation to CO_2 also was determined in same tissues that were assayed for cholesterologenesis. The highest rate of oxidation occurred in adipose tissue and the lowest in liver. Glucose was oxidized most readily by brain and least readily by liver. Mayfield et al. (1966) also observed that adipose tissue had the highest CO_2 production rates from acetate, then brain, and finally liver. Adipose tissue oxidized

Table 6. Relative rates of acetate incorporation into cholesterol and into fatty acids by different tissues from ruminating and nonruminating goats

Tissue	R ¹		NR ²	
	C ³	FA ⁴	C	FA
	%			
Adipose tissue	1.9 ⁵	98.2	0.9	99.2
Small intestine	46.2	53.8	42.7	57.3
Proximal	55.1	44.9	31.6	68.4
Medial	46.2	53.8	34.4	65.6
Distal	42.2	57.8	60.8	39.2
Brain	26.2	73.8	35.0	65.0
Gray	19.1	80.9	27.0	73.0
White	28.8	71.2	38.2	61.8
Liver	58.7	41.3	56.5	43.5

¹R refers to ruminating (hay- and grain-fed) goats.

²NR refers to nonruminating (milk-fed) goats.

³C refers to cholesterol.

⁴FA refers to fatty acids.

⁵Percentage of substrate incorporated into cholesterol and into fatty acids by each tissue.

Table 7. Relative rates of glucose incorporation into cholesterol and into fatty acids by different tissues from ruminating and nonruminating goats

Tissue	R ¹		NR ²	
	C ³	FA ⁴	C	FA
	%			
Adipose tissue	5.1 ⁵	94.9	1.5	98.5
Small intestine	41.8	58.2	47.4	52.6
Proximal	54.7	45.3	38.4	61.4
Medial	35.6	64.4	37.9	62.1
Distal	46.7	53.3	55.9	44.1
Brain	33.9	66.1	31.6	68.4
Gray	10.3	89.7	6.5	93.5
White	46.6	53.4	48.9	51.1
Liver	60.0	40.0	60.0	40.0

¹R refers to ruminating (hay- and grain-fed) goats.

²NR refers to nonruminating (milk-fed) goats.

³C refers to cholesterol.

⁴FA refers to saponified fatty acids.

⁵Percentage of substrate incorporated into cholesterol and into fatty acids by each tissue.

acetate at a rate that was 27 times faster than that in liver. Huang and Kummerow (1976) also monitored glucose and acetate oxidation to CO_2 by specific tissues of mature swine. In contrast to data obtained from goats, glucose was oxidized to CO_2 more readily in liver than in the small intestine, and acetate was oxidized to CO_2 more readily in liver than in adipose tissue.

PART II. FATTY ACID SYNTHESIS IN RUMINATING AND NONRUMINATING GOATS

MATERIALS AND METHODS

Experimental Animals and Tissue Collection

Diets of experimental goats and collection of tissues were the same as those described in Materials and Methods of Section 1.

Assay of Fatty Acid Synthesis

To measure fatty acid synthesis, tissues were incubated as described in Section I. Lipids were extracted from the incubated tissues and washed according to the method of Folch et al. (1957). Hexane then was added to the washed extract to isolate the nonsaponifiable lipids (Pothoven and Beitz, 1973). The residual aqueous phase was acidified to $\text{pH} < 1$ with 12 N HCl. Fatty acids were extracted with hexane and assayed for radioactivity by liquid scintillation counting.

Statistical Analysis

Split plot analysis (Snedecor and Cochran, 1967) was used to analyze the data and P values less than 0.05 were considered to be significant.

RESULTS

Incorporation of [$1\text{-}^{14}\text{C}$] acetate and [$\text{U-}^{14}\text{C}$] glucose into long-chain fatty acids (free plus esterified) was used to measure fatty acid synthesis in ruminating (R) and nonruminating (NR) goats. Lipogenesis was determined on the basis of nmoles of substrate incorporated per two hours of incubation per gram of tissue. Fatty acid synthesis rates also were calculated on the whole organ or total tissue basis. Throughout this paper, total body fatty acid synthesis will refer to the sum of synthesis in each tissue measured.

Rate of Tissue Fatty Acid Synthesis

In adipose tissue, synthesis of fatty acids proceeded at a greater rate in NR goats than in R goats; however, because of the large animal variation, the difference was nonsignificant ($P > 0.05$) when acetate was the precursor (Table 8). Rates in adipose tissue of NR goats were 1.5 and 2.6 times the rates in R goats for glucose and acetate as precursors, respectively. Acetate was preferred over glucose as a precursor for fatty acid synthesis, with synthesis rates being 90 and 50 times greater in R and NR goats, respectively.

Fatty acid synthesis from both acetate and glucose was several-fold less in small intestine than in adipose tissue. Furthermore, rates of incorporation of both substrates were greater in small intestine of R goats than of NR goats. Acetate incorporation was greater than that of glucose in small intestine of both types of goats.

Fatty acid synthesis also was measured in proximal, medial and distal intestinal sections. When goats were fed the hay-grain diet, the lowest rate of synthesis from acetate was at the proximal end; compared

Table 8. Utilization of acetate and glucose for fatty acid synthesis in ruminating and nonruminating goats

Tissue	Acetate and glucose				Glucose			
	R ¹		NR ²		R		NR	
	nmoles substrate incorp./ (2 hr x g tissue)							
Adipose tissue	18,062	± 3,202 ^{3,ab}	26,677	± 6,307 ^{ab}	210	± 148 ^{rb}	548	± 91 ^b
Small intestine ⁴	72.4	± 8.9 ^{ac}	42.7	± 10.7 ^{ac}	18.4	± 7.7 ^{rc}	6.0	± 1.6 ^c
Proximal	35.7	± 2.7 ^a	64.3	± 16.7 ^a	7.3	± 2.1	4.3	± 1.1
Medial	79.0	± 9.1 ^{ra}	32.6	± 7.8 ^a	34.2	± 17.3 ^r	5.9	± 1.6
Distal	102	± 15.0 ^{ra}	31.4	± 7.7 ^a	13.6	± 3.6	7.8	± 2.0
Brain ⁴	18.6	± 4.6 ^d	13.4	± 1.6 ^d	8.2	± 3.1 ^c	10.4	± 2.0 ^c
Gray	11.0	± 1.7	8.4	± 1.2	7.8	± 2.0	11.6	± 3.1
White	26.2	± 7.6 ^a	18.3	± 2.0 ^a	8.6	± 4.1	9.2	± 0.8
Liver	9.3	± 2.9 ^{rae}	2.7	± 0.9 ^{ae}	0.4	± 0.2 ^d	0.2	± 0.1 ^d

¹R refers to ruminating (hay- and grain-fed) goats.

²NR refers to nonruminating (milk-fed) goats.

³Mean ± SEM.

⁴Averages were calculated assuming equal weights of each tissue subdivision.

^rSignificant difference ($P < 0.05$) compared with NR goats (within tissue and substrate comparisons).

^aSignificant difference ($P < 0.05$) compared with glucose substrate (within tissue and diet comparison).

^{b,c,d,e}Values in the same column not sharing a common superscript letter are significantly different ($P < 0.05$). Comparisons were not made for individual sections of small intestine and brain.

with that in proximal end, rate in medial section was double, and in distal end, triple. Glucose was used most effectively for fatty acid synthesis in the medial portion of small intestine of R goats, with the proximal and distal portions having a rate only one-fifth and one-third, respectively, of the rate in the medial section. In NR goats, the greatest fatty acid synthesis rate from acetate occurred at the proximal end of the small intestine; the medial and distal portions synthesized fatty acids at a rate only one-half of that in the proximal section. Compared with incorporation of acetate, glucose was used at a negligible rate throughout the length of the intestine.

Brain of R and NR goats showed similar rates of fatty acid synthesis from either glucose or acetate. In both groups of goats, however, the brain white matter synthesized fatty acids from acetate twice as fast as did gray matter; no difference was noted in glucose incorporation. In comparison to that in other tissues, liver of R and NR goats synthesized fatty acids at a negligible rate.

Whole Organ or Total Tissue Fatty Acid Synthesis

Adipose tissue was found to make the greatest contribution to total fatty acid synthesis (Table 9). With acetate as a precursor, adipose tissue synthesized 99% of all fatty acids in both R and NR goats. Liver, brain and small intestine together contributed less than one percent of total fatty acid synthesis; however, intestinal lipogenesis was slightly favored over that in liver and brain. With glucose as precursor, some tissue differences in lipogenesis between R and NR goats were evident. In R goats, adipose tissue synthesized 84% of the total fatty acids; small intestine contributed 13%, and brain, 3%. In NR goats, 98.4% of total fatty acid synthesis occurred in adipose tissue. Of the remaining fatty

Table 9. Synthesis of fatty acids in whole organs of ruminating and nonruminating goats¹

Tissue	Acetate and glucose		Glucose	
	R ²	NR ³	R	NR
Adipose tissue	99.1 ± 0.9 ⁴	99.8 ± 0.5	84.0 ± 5.7 ^r	98.4 ± 0.0
Small intestine	0.7 ± 0.2	0.1 ± 0.0	12.6 ± 3.6 ^r	0.5 ± 0.1
Brain	0.1 ± 0.0	0.0 ± 0.0	3.0 ± 0.2	1.0 ± 0.4
Liver	0.1 ± 0.0	0.0 ± 0.0	0.3 ± 0.1 ^r	0.1 ± 0.0

¹Data are expressed as percentage of total synthesis in all four tissues.

²R refers to ruminating (hay- and grain-fed) goats.

³NR refers to nonruminating (milk-fed) goats.

⁴Mean ± SEM.

^rSignificant difference (P < 0.05) compared with NR goats (within tissue and substrate comparisons).

acids synthesized, small intestine contributed 0.5%, and the brain, 1%. The liver of both types of goats contributed negligibly to total lipogenesis.

Adipose tissue contributed greater than 98% of the endogenously synthesized fatty acids in the four-month-old goat when either glucose or acetate was used as precursor. Small intestine, brain and liver each contribute minor amounts of long-chain fatty acids when compared with that of adipose tissue.

Fatty acid synthesis from acetate also was determined for tissues which were incubated in glucose-free media. In all tissues observed there were significant decreases in incorporation rate of acetate into fatty acids when glucose was omitted (Table 10). The most dramatic decrease occurred with adipose tissue.

Utilization of Alternate Precursors

Mevalonate and β -hydroxybutyrate also were tested as substrates for fatty acid synthesis in adipose tissue and distal small intestine. Both tissues of NR goats used mevalonate for lipogenesis more readily than did R goats. Both acetate and glucose were used more readily than was mevalonate by adipose tissue in both tissues of R goats. In NR goats, adipose tissue used glucose four times as well as mevalonate (550 vs. 130 nmoles/[g x 2 hr]), and acetate 200 times as well for lipogenesis (26,000 vs. 130). The small intestine of NR goats incorporated mevalonate into fatty acid at a rate that was three times that of acetate and fifteen times that of glucose. R goats used mevalonate and acetate equally well for intestinal lipogenesis and used glucose only one-tenth as well.

Table 10. Acetate incorporation (in absence of exogenous glucose) into cholesterol in ruminating and nonruminating goats

Tissue	Rate		Percentage	
	R ¹	NR ²	F	NR
	nmoles substrate incorp./ (2 hr x g tissue)		% ³	
Adipose tissue	401 ± 60 ⁴	1,494 ± 495	2.2	5.6
Small intestine (medial)	20.6 ± 4.9	18.1 ± 7.7	26.1	55.6
Brain				
Gray	2.8 ± 1.5	2.7 ± 1.4	25.4	32.1
White	5.4 ± 1.9	6.0 ± 0.6	20.6	32.7

¹R refers to ruminating (hay- and grain-fed) goats.

²NR refers to nonruminating (milk-fed) goats.

³Calculated as a percentage of the rate of acetate incorporation in the presence of glucose.

⁴Mean ± SEM.

β -Hydroxybutyrate was incorporated into fatty acid by adipose tissue at a rate that was significantly lower than that for acetate (315 vs. 18,000). but similar to that for glucose (315 vs. 210). Lower rates of lipogenesis from β -hydroxybutyrate existed in adipose tissue of NR goats than in that of R goats; the rate in the small intestine of NR goats was two-thirds of that in R goats.

DISCUSSION

Previous studies involving ruminants have shown that adipose tissue plays a major role in fatty acid synthesis. Liver, which is a major lipogenic tissue in many nonruminating animals, is a minor organ for synthesis of fatty acids (Ingle et al., 1972a and 1972b; Hood et al., 1972; Bauman, 1974). The results presented in my study involving R goats are in accord with these reported studies. Also, goats maintained on a goat-milk diet (nonruminating condition) showed a high rate of lipogenesis in adipose tissue and a low rate in hepatic tissue.

When acetate was used as a substrate, my data suggest that R and NR goats synthesize 99% of endogenously-synthesized fatty acids in adipose tissue. Although glucose is incorporated to a lesser degree than acetate by most tissues, it is incorporated most readily by adipose tissue. In NR goats, 98% of total lipogenesis from glucose occurred in adipose tissue; in R goats, the proportion contributed by adipose tissue was reduced to 84%. With either substrate and either diet, fatty acid synthesis in liver was less than 1% of that in the other tissues.

The high values obtained in my study for acetate incorporation into fatty acids by adipose tissue are representative of information gathered from a number of studies. Mayfield et al. (1966) showed that in sheep, the primary site of acetate oxidation to CO_2 and incorporation into fatty acids was adipose tissue. In 1967, Hanson and Ballard studied lipogenesis in adipose tissue of cattle and sheep, using acetate and glucose as precursors. Incorporation of acetate into fatty acids was much greater than that for glucose. It was shown that one of the key enzymes involved in synthesis of fatty acid from acetate (acetyl CoA synthetase) occurred

at a relatively high concentration in ruminant adipose tissue, whereas in nonruminants such as rats, it was at a lower concentration.

Ingle, et al. (1972a) also measured rates of lipogenesis from acetate and glucose and found dramatic differences for ruminant adipose tissue. The rate of adipose tissue lipogenesis from acetate was very high in sheep and cattle, but that from glucose was low. Also, they calculated the percentage of synthesis from acetate per tissue type in market lambs and found liver and adipose tissue to have lipogenesis rates of 5% and 92%, respectively.

In my study, the rates of fatty acid synthesis from acetate were compared to rates of synthesis from glucose. Acetate was incorporated into fatty acids much more readily in all tested tissues. When glucose was incubated with adipose tissue from R and NR goats, the rate of lipogenesis was one-hundredth and one-fiftieth of the rate of acetate incorporation, respectively. Ruminating goats, however, synthesized fatty acids at a rate that was two-thirds of the NR goats' rate in adipose tissue. With any precursor or diet combination, rate of hepatic lipogenesis was low. Therefore, our results are in agreement with those of others.

It has been postulated by Favarger (1965) and Dietschy and McGarry (1974) that problems may exist when one uses labeled acetate to determine hepatic lipogenesis rates in nonruminants because of isotope dilution by acetyl CoA produced from endogenous precursors. This effect is minimal in my experiments because endogenous acetyl CoA produced via mitochondrial reactions does not equilibrate readily with the cytosol acetyl CoA pool. The lack of citrate cleavage enzyme and malic enzyme in ruminant tissues reduces the quantitative important of transfer of mitochondrial acetyl CoA to the cytosol where fatty acid synthesis occurs (Hanson and Ballard, 1967;

Bauman et al., 1970; Ingle et al., 1972a). In ruminants, mitochondrially-produced acetyl CoA seems not incorporated into fatty acids as efficiently as in nonruminants.

Small intestine contributed less than 1% to total body lipogenesis when acetate was used as precursor. This observation agrees with that of Ingle et al. (1972b) who demonstrated that in vivo acetate incorporation into fatty acids by sheep intestine was only 3 to 4% of total fatty acid synthesis. In my study, glucose incorporation into fatty acids by the small intestine was also low in both R and NR goats. In work similar to mine, Romsos (1971) studied lipogenesis in swine intestine from acetate. It was shown that intestinal fatty acid synthesis rates were highest in the proximal section of the intestine where the greatest amount of lipid absorption occurs. Reasons for intestinal fatty acid synthesis have not yet been established. It has been speculated that lipogenesis is necessary for the structural needs of the cell (Tame and Dils, 1967). If this is correct, then the higher rate of synthesis found in R goat is perhaps due to an increased intestinal motility and an increased "abrasion" and cell turnover.

In the goat brain, little fatty acid synthesis was expected because the brain is almost completely formed by the fourth month after birth. In this study, both precursors were incorporated into fatty acids at a comparatively low rate in both R and NR goats. Incorporation was slightly higher in the brain of R goats that synthesized 3% of the body fatty acids from glucose, whereas NR goats synthesized 1% in brain.

In other tissues studied, acetate is used more readily than glucose for fatty acid synthesis. In the brain, the rates of incorporation

of each substrate are similar. This can be expected because glucose penetrates the blood-brain-barrier more readily than does acetate and is the predominant energy source for brain tissue (Smith, 1967). This conclusion was supported by data on CO_2 production (Table 5). A higher rate of CO_2 production was found in the brain when glucose was used as a precursor as opposed to acetate.

Another important observation was that brain white matter showed a higher lipogenic capacity than brain gray matter when acetate was used as a precursor, and an equal capacity with glucose. This is to be expected because brain white matter contains the nervous system's myelin sheaths which have greater amounts of supportive lipids, whereas brain gray matter consists largely of glial cells which contain much lesser amounts of lipids (McIlwain, 1966).

Adipose tissue and small intestine were used to compare incorporation of β -hydroxybutyrate and mevalonate into fatty acids. In adipose tissue of both R and NR goats, acetate was the major fatty acid precursor, then β -hydroxybutyrate, glucose and mevalonate in decreasing order. Acetate was incorporated 180 times faster than mevalonate, whereas β -hydroxybutyrate was incorporated 60 times faster than mevalonate in the NR goats. In the R goats, acetate was incorporated 90 times faster than mevalonate and 60 times faster than β -hydroxybutyrate.

Incorporation of β -hydroxybutyrate into fatty acids has been shown to occur in mammary tissue from cattle and rats (Kinsella, 1969). A number of other researchers (Palmquist et al., 1969; Bines and Brown, 1968; Smith and McCarthy, 1969) concluded that only one β -hydroxybutyrate molecule is utilized in any one of the fatty acids synthesized de novo in ruminant mammary tissue and, consequently, acetate would be incorporated more

extensively. Kinsella (1969) has shown that in mammary tissue from lactating cattle acetate is incorporated to a greater degree than β -hydroxybutyrate. Although our study on adipose tissue shows a greater difference in acetate and β -hydroxybutyrate incorporation than that of Kinsella on mammary tissue, both tissues show a strong preference for acetate as a fatty acid precursor.

The low rate of mevalonate incorporation in fatty acids by adipose tissue is not unusual, because the majority of the mevalonate present would be predicted to be used for steroid biosynthesis (Dempsey, 1974). In the intestine, the predominant fatty acid precursor was mevalonate, followed by acetate, β -hydroxybutyrate and glucose. Recently, Edmond and Popják (1974) presented evidence for the existence of a pathway in which intermediates arising from mevalonate can be converted to HMG-CoA, ketone bodies and acetyl CoA in addition to squalene and cholesterol. This pathway, termed the trans-methylglutaconate shunt, is thought to be able to shunt up to 20% of the mevalonate away from steroid biosynthesis. This is the most probable pathway for mevalonate incorporation into fatty acids. My data suggest that this pathway has greater flux for fatty acid synthesis than that of pathways for acetate and glucose incorporation into fatty acids in the small intestine but not in adipose tissue and brain.

To summarize, adipose tissue is much superior to intestine in terms of fatty acid synthesis and acetate is used much more readily as a precursor than are mevalonate, β -hydroxybutyrate or glucose.

SUMMARY

The main goals of this work were to (1) compare rates of cholesterol and fatty acid synthesis in tissues of ruminating (R) and nonruminating (NR) goats, (2) determine what percentage of total cholesterol or total fatty acids are synthesized by each tissue analyzed and (3) compare rates of synthesis of cholesterol and fatty acids from acetate or glucose. The long-term objective of this study was to elucidate the mechanism and control of cholesterol and fatty acid synthesis and, consequently, to increase our understanding of the causes of atherosclerosis.

The most significant conclusions of this study can be summarized as follows:

1. Adipose tissue is the primary tissue responsible for cholesterol and fatty acid synthesis in the R and NR goat.
2. Liver is a minor contributor of cholesterol and fatty acids in R and NR goats.
3. Acetate is preferred over glucose as a lipid precursor by most tissues from R and NR goats.
4. Ruminating goats have higher rates of cholesterogenesis in small intestine and in liver than NR goats.
5. Acetate is oxidized to CO_2 most readily by adipose tissue, whereas glucose is oxidized most readily by the brain.

More work is needed to increase our understanding of in situ cholesterol and fatty acid synthesis. Possible areas that should be examined in the future include:

1. Examination of all possible sources of cytosolic acetyl CoA, including that derived from mitochondria.

2. Elucidation of the regulation of flux of carbon to cholesterol and fatty acids.
3. A study in which uptake of cholesterol and fatty acids by different tissues is monitored to better understand lipid transport, storage and atherosclerosis.
4. A study of possible dietary effects on cholesterol and fatty acid synthesis in different tissues.

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APPENDIX

Appendix Table 1. Cholesterol synthesis in tissues of nonruminating goats

Tissue	Substrate	Goat number				
		213	228	231	238	239
Brain (gray matter)	glucose	1.1	0.5	0.8	0.8	0.9
Brain (white matter)	glucose	12.1	4.9	7.8	10.6	8.1
Brain (gray matter)	acetate	5.1	6.8	0.7	1.3	1.4
Brain (white matter)	acetate	8.1	7.9	9.7	12.0	18.9
Liver	glucose	0.1	0.5	0.5	0.3	0.0
Liver	acetate	2.0	8.7	2.8	2.6	1.4
Adipose tissue	glucose	1.4	18.9	3.0	12.6	6.6
Adipose tissue	acetate	32.9	429.3	123.5	263.4	286.5
Duodenum	glucose	1.4	6.5	2.9	1.9	0.6
Duodenum	acetate	16.7	59.3	42.4	19.9	10.1
Jejunum	glucose	6.0	5.1	4.1	2.2	0.8
Jejunum	acetate	12.0	20.1	32.0	9.4	12.2
Ileum	glucose	4.8	20.1	8.5	11.2	4.9
Ileum	acetate	8.6	95.1	19.2	79.1	41.8
Brain (gray matter)*	acetate	**	0.4	5.8	3.0	0.0
Brain (white matter)*	acetate	**	3.6	3.1	8.6	6.1
Adipose tissue*	acetate	**	82.9	18.6	37.8	50.0
Jejunum*	acetate	**	19.6	24.6	6.3	10.4

*Glucose was not included in the incubation media.

**Data was not collected.

Appendix Table 2. Cholesterol synthesis in tissues of ruminating goats

Tissue	Substrate	Goat number				
		214	220	235	239	261
Brain (gray matter)	glucose	1.4	1.6	0.5	0.5	0.4
Brain (white matter)	glucose	9.3	4.0	10.1	7.2	7.0
Brain (gray matter)	acetate	3.6	0.8	5.3	2.0	1.3
Brain (white matter)	acetate	10.0	11.4	13.9	8.4	9.5
Liver	glucose	0.3	1.0	0.0	1.5	0.2
Liver	acetate	9.7	23.9	5.9	21.0	5.4
Adipose tissue	glucose	27.8	15.4	3.7	3.8	5.7
Adipose tissue	acetate	114.5	425.3	837.5	210.5	119.3
Duodenum	glucose	9.4	7.8	9.2	13.9	3.8
Duodenum	acetate	36.4	27.1	69.2	48.4	38.1
Jejunum	glucose	13.1	9.6	42.6	12.0	17.6
Jejunum	acetate	47.9	57.2	66.5	58.2	109.3
Ileum	glucose	13.3	8.5	10.2	23.7	3.9
Ileum	acetate	61.0	67.6	73.0	111.5	62.5
Brain (gray matter)*	acetate	**	0.0	1.0	0.4	0.1
Brain (white matter)*	acetate	**	5.1	7.8	3.6	8.8
Adipose tissue*	acetate	**	24.9	28.5	49.5	19.9
Jejunum*	acetate	**	29.7	55.1	26.2	65.6

*Glucose was not included in the incubation media.

**Data was not collected.

Appendix Table 3. Fatty acid synthesis in tissues of nonruminating goats

<u>Tissue</u>	<u>Substrate</u>	<u>Goat number</u>				
		213	228	231	238	259
Brain (gray matter)	glucose	11.2	12.9	22.4	8.2	3.3
Brain (white matter)	glucose	10.4	8.6	12.5	11.7	2.9
Brain (gray matter)	acetate	8.0	11.7	10.5	6.1	5.5
Brain (white matter)	acetate	19.3	20.6	24.3	14.5	13.2
Liver	glucose	0.8	0.6	0.0	0.6	0.4
Liver	acetate	2.6	2.7	6.1	0.4	1.8
Adipose tissue	glucose	167.2	532.0	321.3	1,016.5	705.9
Adipose tissue	acetate	6,267.0	31,600.4	20,125.7	31,813.4	43,577.3
Duodenum	glucose	2.1	8.3	2.9	5.6	2.8
Duodenum	acetate	15.9	101.6	103.3	49.8	51.2
Jejunum	glucose	9.3	7.6	8.5	3.3	0.7
Jejunum	acetate	21.7	31.8	60.6	34.3	14.8
Ileum	glucose	5.8	15.7	3.7	7.3	6.3
Ileum	acetate	11.6	17.0	53.5	33.1	41.9
Brain (gray matter)*	acetate	**	2.0	7.4	0.1	1.1
Brain (white matter)*	acetate	**	5.3	6.7	7.3	4.7
Adipose tissue*	acetate	**	797.6	219.7	2,463.6	496.3
Jejunum*	acetate	**	15.7	40.0	3.5	13.1

*Glucose was not included in the incubation media.

**Data was not collected.

Appendix Table 4. Fatty acid synthesis in tissues of ruminating goats

Tissue	Substrate	Goat number				
		214	220	235	239	261
Brain (gray matter)	glucose	9.9	13.5	9.1	2.0	4.6
Brain (white matter)	glucose	9.5	10.3	6.7	10.2	6.3
Brain (gray matter)	acetate	7.3	15.4	13.4	12.1	6.7
Brain (white matter)	acetate	14.9	25.9	17.6	55.8	16.6
Liver	glucose	0.5	0.2	1.2	0.3	0.0
Liver	acetate	4.2	20.1	3.9	8.8	9.4
Adipose tissue	glucose	191.6	227.5	41.1	47.9	543.3
Adipose tissue	acetate	8,493.3	13,680.1	22,566.0	26,622.3	18,952.8
Duodenum	glucose	14.4	8.8	3.8	7.4	2.1
Duodenum	acetate	39.1	37.5	36.9	40.0	24.9
Jejunum	glucose	20.1	103.3	17.9	17.1	12.6
Jejunum	acetate	106.2	50.2	72.3	80.4	85.9
Ileum	glucose	18.2	11.5	10.0	24.8	3.3
Ileum	acetate	82.5	86.6	98.0	161.7	84.2
Brain (gray matter)*	acetate	**	2.0	1.3	7.8	0.1
Brain (white matter)*	acetate	**	10.6	2.4	5.8	2.7
Adipose tissue*	acetate	**	238.9	345.7	487.5	535.0
Jejunum*	acetate	**	13.5	34.5	13.4	20.8

*Glucose was not included in the incubation media.

**Data was not collected.

Appendix Table 5. Glucose and acetate oxidation to CO₂ in tissues of nonruminating goats

<u>Tissue</u>	<u>Substrate</u>	<u>Goat number</u>		
		231	228	259
Brain (gray matter)	glucose	2,681	2,854	2,790
Brain (white matter)	glucose	1,092	1,931	1,826
Brain (gray matter)	acetate	1,793	797	355
Brain (white matter)	acetate	1,374	738	785
Liver	glucose	238	237	225
Liver	acetate	4,830	661	643
Adipose tissue	glucose	610	2,526	1,957
Adipose tissue	acetate	6,957	6,066	10,281
Duodenum	glucose	552	339	423
Duodenum	acetate	3,440	778	1,444
Jejunum	glucose	1,285	234	342
Jejunum	acetate	5,585	42	1,326
Ileum	glucose	585	313	670
Ileum	acetate	3,125	1,528	2,107
Brain (gray matter)*	acetate	3,775	848	160
Brain (white matter)*	acetate	2,227	468	0.0
Adipose tissue*	acetate	3,939	2,032	6,163
Jejunum*	acetate	6,504	303	1,863

*Glucose was not included in the incubation media.

Appendix Table 6. Glucose and acetate oxidation to CO₂ in tissues of ruminating goats

<u>Tissue</u>	<u>Substrate</u>	<u>Goat number</u>		
		231	228	259
Brain (gray matter)	glucose	2,333	1,945	984
Brain (white matter)	glucose	1,264	1,262	903
Brain (gray matter)	acetate	308	717	575
Brain (white matter)	acetate	1,888	845	122
Liver	glucose	163	416	351
Liver	acetate	2,920	1,424	1,890
Adipose tissue	glucose	812	840	815
Adipose tissue	acetate	14,964	9,576	6,587
Duodenum	glucose	683	891	544
Duodenum	acetate	4,785	2,594	4,071
Jejunum	glucose	1,089	646	912
Jejunum	acetate	3,509	3,376	2,439
Ileum	glucose	634	668	401
Ileum	acetate	3,526	3,456	1,920
Brain (gray matter)*	acetate	931	404	470
Brain (white matter)*	acetate	630	167	340
Adipose tissue*	acetate	3,481	5,646	3,691
Jejunum*	acetate	3,573	2,470	2,590

*Glucose was not included in the incubation media.

Appendix Method 1. Procedure for incubation and isolation of cholesterol and fatty acids from goat tissue

1. Incubate tissue at 37° for 2 hr under 95% O₂ - 5% CO₂ at 90-100 strokes/min.
2. After 2 hr introduce 0.1 ml. 25% KOH on paper and add 0.5 ml 1,0 N H₂SO₄ to the solution.
3. Shake for 45 minutes.
4. Transfer filter paper no. 4 (KOH soaked) to vials and add 1 ml distilled water; then add counting solution.
5. Put tissue on filter paper and wash 3Xs with normal saline.
6. Transfer to screw cap test tubes and add 15 ml. C-M* (2:1).
7. Shake overnight on wrist action shaker.
8. Transfer extracts to previously graduated test tubes and make up to 20 ml with C-M (2:1).
9. Add 4 ml of 0.73 NaCl and mix well with automatic stirrer.
10. Centrifuge for 10 minutes at 1500 RPMs.
11. Remove upper layer with suction.
12. Add 3 ml of pure solvent upper phase (C-M .29% saline - at a ratio of 3:48:47) and stir on a vortex genie.
13. Centrifuge and remove the supernatant.
14. Repeat steps 13 and 14 two more times.
15. Evaporate extract to dryness under a steady flow of N₂ (to retard oxidation).

*Denotes chloroform-methanol mixture.

16. Add 6 ml of 3% methanolic KOH (15 g KOH in 7 ml H₂O added to 485 ml methanol).
17. Reflux sample at 80-85° for 120 minutes in order to saponify it. Then add 4 ml of H₂O and allow the sample to cool.
18. Carry out three extractions with 7, 7 and 3.5 ml of pet ether consecutively. Combine all extracts in a scintillation vial and allow to dry under air. Count using toluene scintillation fluid.