

16. Energy Metabolism

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INTRODUCTION

The liver serves as an intermediary between the dietary sources of energy and the extrahepatic tissues that are the main users of energy. In that capacity the liver receives, by way of the portal circulation, the various small molecules arising from digestion, sorts them for metabolism and storage, and distributes some to the peripheral circulation for use by other tissues. To carry out those functions and to maintain a structural apparatus for their performance, the liver extracts a significant portion of the nutrients to provide energy for its own macromolecular syntheses, transport of materials in and out of liver cells, and metabolic conversions within the cells.

The liver also receives metabolites transported by the blood from other tissues. Thus in relation to provision of energy, the liver obtains fatty acids and glycerol from adipose tissues, lactate and pyruvate from skeletal muscle and blood cells, alanine and certain other amino acids from skeletal muscle, and branched-chain α -keto acids from skeletal muscle, where they arise by transamination of leucine, isoleucine, and valine (1,2). In turn, the liver exports two principal substrates that can be oxidized in peripheral tissues to provide energy. The first is glucose; this arises by glycogenolysis of stored glycogen and by gluconeogenesis from lactate, pyruvate, glycerol, propionate, and alanine. Many other amino acids whose carbon chains can give rise to pyruvate, oxaloacetate, fumarate, malate, succinate, or α -ketoglutarate can contribute to production of glucose by gluconeogenesis. The second substrate made by the liver for transport to other tissues is acetoacetate (or its reduced derivative, 3-hydroxybutyrate). Acetoacetate arises from acetyl-coenzyme A (CoA) coming mainly from the oxidation of fatty acids transported to the liver from adipose tissue. The liver also synthesizes storage lipids in the form of triacylglycerols and phospholipids; these are packaged in lipoproteins and delivered to the blood for use in peripheral tissues. The scheduling of synthesis and transport of these substrates is exquisitely regulated by hormones and coordinated with the amounts and nature of fuels from the diet, with the length of time after feeding, and with the quantity of glucose available in relation to the requirements of tissues that have an obligate need for glucose. Those tissues are the blood cells, especially erythrocytes, retina, and renal medulla. Thus the liver is sensitive to the kinds of fuels needed by specific tissues under different physiological conditions and, in cooperation with other tissues, is programmed to provide those fuels. Recently a review has appeared describing the mechanisms by which the liver provides a constant supply of substrates for oxidation in other tissues (3).

In addition to manufacturing and exporting fuels that can be oxidized directly for energy in other tissues, the liver provides critical compounds to be used by those tissues in enabling and permissive capacities relative to the release and storage of energy arising from substrate oxidation. Thus the liver carries out the final steps in the synthesis of carnitine and creatine, both of which are sent to the peripheral circulation to be concentratively absorbed by target organs that utilize them in their energy machinery (4--6). Carnitine, 4-*N*-trimethyl-3-hydroxy-aminobutyric acid, is synthesized as follows (7--10):

L-Lysine \rightarrow protein peptidyl lysine

S-adenosyl-

L-methionine

protein peptidyl 6-*N*-trimethyl-L-lysine \rightarrow free
6-*N*-trimethyl-L-lysine \rightarrow 3-hydroxy-6-*N*-trimethyl-
L-lysine 4-*N*-trimethylaminobutyraldehyde
 \rightarrow 4-*N*-trimethylaminobutyric acid \rightarrow L-carnitine

The final hydroxylation step in humans is performed in the liver and kidneys (11,12). Some of the resulting carnitine is used both in mitochondria and peroxisomes of hepatocytes for the transport of fatty acids across

membranes, but most is exported for use in other tissues that metabolize fatty acids for energy, that is, skeletal and cardiac muscle. Deficiency of carnitine can result in poor performance of muscle, since it effectively deprives the tissue of capacity to oxidize fatty acids (5,13).

The liver is also the site of the final synthesis of creatine, which occurs by methylation of guanidinoacetate by S-adenosylmethionine (6). Although the liver has little creatine kinase (14), and therefore hardly uses creatine for its own energy balance, it exports creatine to tissues such as skeletal muscle, heart, and brain. These tissues have mechanisms for concentrative absorption of creatine from extracellular fluid and use creatine as an acceptor of the terminal high-energy phosphate group of ATP as governed by the action of creatine kinase. If the liver were not to make creatine, the entire energy balance of those tissues would be upset, not only because a reserve of high-energy phosphate would be diminished, but because the nature of the adenine nucleotide pool would be affected. The presence of creatine helps regulate the ATP:ADP ratio, which in turn affects the oxidative capacity of a tissue.

The liver makes other compounds for export that, in one way or another, affect energy release and utilization in various tissues; these are not considered in detail here. This chapter is primarily concerned with the nature of substrates that the liver uses to provide energy for its many transport and biosynthetic functions, the metabolic pathways open to these substrates, the interrelationships among the pathways, and finally, the regulation of those pathways to allow the liver a choice among them in relation to the physiological state of the whole organism. We also examine the several ways in which the liver provides specific fuels for different extrahepatic tissues according to their requirements; this includes consideration of the signals to the liver that make it sensitive to the needs of other tissues.

NATURE OF FUELS PROVIDED TO THE LIVER BY THE INTESTINE

We first discuss some general considerations about the nature of diets that provide the fuels. Human diets vary depending on economic factors, climate, geographical location, development of agriculture and other technologies, and the cultural habits and energy requirements of different peoples. Our examples are chosen largely from the diets eaten in the United States, Canada, and many parts of Europe, where the level of protein is higher than in the diets of peoples in tropical areas and the level of cereal carbohydrates (mainly starches) is lower. These people ingest a large percentage of their carbohydrate in the form of highly refined sugars such as sucrose; in fact, the annual per capita consumption of refined sugars and syrups in the United States and Great Britain averages about 115 pounds, as compared to about 146 pounds of cereal carbohydrate (15). The following discussion deals largely with diets of that kind.

If one assumes a diet that provides 3000 kilocalories¹ per day, the average adult working person in the United States partitions the provision of energy as approximately 13% to 15% from proteins, 40% from carbohydrates, and 40% from fats. Approximately 7% of calories is derived from ethanol (16), a consideration of importance for the biochemistry of the liver (17). (In a significant number of people, the percentage of calories from ethanol is much higher.) Translated in terms of amount of nutrients provided, the statistically average person daily ingests approximately 100 g protein, 130 g fat, 300 g carbohydrate, and 30 g ethanol. Taking into account the further partitioning of dietary carbohydrate, that individual consumes approximately 120 g sucrose, of which 60 g appears as fructose. Thus the liver of the average person must metabolize two special compounds, fructose and ethanol, from which a significant amount of energy may be derived.

Because of the considerable current interest in nondigestible fibrous components of the diet, we should mention another polymeric form of fructose consumed by humans in a low but nevertheless significant amount. This is the plant polymer of fructose called inulin, made up of fructose units in 2,1-linkages; humans consume both inulin and its partially degraded products known collectively as oligofructose. Oligofructose, formed enzymatically, consists of polymers varying from 2 to 20 monomeric units. The biochemistry of these materials can be summarized as follows (18). The intake of inulin and its derived oligofructose is about 2 to 12 g per day per person in Western Europe and 2 to 8 g per day per person in North America. Most of this is metabolized by *Bifidobacteria* in the gut, and end products are carbon dioxide, acetate, pyruvate, lactate, and ethanol. Lactate and pyruvate are fermented further to short-chain fatty acids, so that about 80% of the carbon chains are converted to acetate, propionate, and butyrate. These substances, absorbed through the intestinal wall, are metabolized both in intestinal cells (especially butyrate) and in skeletal muscle and the liver. The energy obtained in the human being by the pathways involved is estimated to be about 1 kcal per gram of oligofructose consumed; this can be compared to the value of 4 kcal per gram obtained from the intake of 1 g of fructose, any other common hexose, and of course sucrose.

Foodstuffs are ingested intermittently; consequently, the metabolism of the liver has evolved in relation to that circumstance and is characterized by poorly understood circadian rhythms and diurnal cycles (19,20).

After digestion and absorption of foodstuffs, the liver, through the portal circulation, is presented with a mixture of amino acids, monosaccharides, short-chain fatty acids, and occasionally, ethanol. The liver is able to process most of the amino acids through a combination of transamination and oxidative deamination, but poorly transaminates the branched-chain amino acids leucine, isoleucine, and valine (21). In several situations, such as in refeeding after starvation, splanchnic uptake of those amino acids is incomplete (22). They are sent primarily into the peripheral circulation, from which they are extracted, especially by skeletal muscle. In muscle they can be used in protein synthesis or, under conditions of short supply of glucose, transaminated with pyruvate to form the corresponding branched-chain α -keto acids and alanine (21). Again, under conditions of glucose lack, those acids may be sent to the liver to be processed into energy sources for the liver itself and for other tissues; the processes involved are considered subsequently.

In addition to long-chain fatty acids, dietary lipids contain a variety of short-chain fatty acids. When released by lipolytic digestion, these are relatively easily absorbed into the intestinal cells, having the advantage of smaller molecular weight and greater water solubility as compared with long-chain fatty acids. Resynthesis of triacylglycerols from monoacylglycerols occurs in the intestinal cells, with preference given to the incorporation of long-chain fatty acids. Accordingly, the short-chain acids remain free, are easily transferred to the portal circulation, and are carried to the liver (23). Immediately after digestion of a meal containing fats, the liver is presented primarily with short-chain fatty acids and utilizes them for its energy metabolism through oxidation in the mitochondrial fatty acid oxidation system. Nevertheless, the liver ultimately receives some of the long-chain fatty acids released by digestion, but the process is circuitous. The triacylglycerols reconstituted in the intestinal absorbing cells are incorporated into chylomicrons together with cholesterol esters and phospholipids and sent into the lymph (24,25). The lymph empties into the circulation at the thoracic duct, and the chylomicrons are then circulated throughout the organism. Chylomicrons presented to the adipose tissue are acted on by an endothelial lipoprotein lipase (not to be confused with the hormone-sensitive lipase in adipocytes), releasing long-chain fatty acids (which originated in the dietary lipids) and diacylglycerols (24,25).

The latter may be taken into adipocytes where they can be resynthesized into triacylglycerols, but the fatty acids are transferred to plasma albumin and carried in the blood to the various tissues. Some of the fatty acid is extracted by the liver, so that the organ eventually does derive some fraction of long-chain fatty acids from the dietary lipids. Furthermore, the remnants of chylomicrons, which still contain some triacylglycerols, are also carried by the blood to the liver where hepatic triacylglycerol lipase may provide an additional fraction of the dietary long-chain fatty acids. However, most long-chain fatty acids are brought to the liver after lipolysis of triacylglycerols stored in adipocytes (26,27).

USE OF OXYGEN BY THE LIVER FOR ENERGY FUNCTIONS

The hepatoportal system is highly aerobic. Constituting only about 4% of the total body weight, it receives about 28% of the total blood flow and consumes about 20% of the total oxygen used by the human organism (28).

In the basal state, a 70-kg man uses about 400 liters of oxygen per day (29), of which about 80 liters are consumed by the liver. This occurs in relation to the 340 to 360 kcal of energy expended by the liver under basal conditions (20% of a total of 1800 kcal used by the whole organism) (30). This matter is considered further in a recent review of fuel utilization (26).

The oxygen is used primarily for the oxidation of fuels, most of which results in the preservation of free energy as the high-energy pyrophosphate bonds of ATP; the standard free energy of ATP is 7.3 kcal/mole, or 30.5 kJ/mole. Perhaps about 90% of the oxygen consumed by the liver is used in reactions that can be poisoned by cyanide, constituting the so-called cyanide-sensitive respiration, most of which is related to the mitochondrial electron transport system or respiratory chain in which cytochrome oxidase is the iron hemoprotein sensitive to cyanide.

Cyanide-insensitive respiration is principally associated with enzymes present in peroxisomes or microbodies; these enzymes utilize flavin nucleotides as coenzymes, the oxidation-reduction potentials of which are modified so that they can couple directly with molecular oxygen (31); hydrogen peroxide is formed instead of water, as produced in the mitochondrial respiratory chain. Examples of such flavin nucleotide-containing enzymes are xanthine oxidase, uricase, D-amino acid oxidase, and fatty acyl-CoA oxidase. Hydrogen peroxide produced in peroxisomes is metabolized by the action of catalase, which, in the absence of a suitable oxidizable substrate, catalyzes the release of molecular oxygen; in the presence of such a substrate (e.g., ethanol or methanol), catalase causes reduction of hydrogen peroxide (32).

Under most conditions, the liver obtains the largest fraction of its ATP through the oxidation of fatty acids; considerably less is derived from the oxidation of pyruvate formed in glycolysis or from lactate brought from skeletal muscle and blood cells. If the liver were oxidizing only fatty acids, its theoretical respiratory quotient (RQ), defined as the ratio of volumes of carbon dioxide produced to volumes of oxygen consumed, would be approximately 0.71. In reality, that figure would be modified upward if the liver were simultaneously synthesizing

fatty acids from glucose and glucose by gluconeogenesis. In fact, energy derived from oxidation of fatty acids is used for driving gluconeogenesis.

Glycogen stored in the liver is used primarily to provide glucose to the blood for distribution to tissues that require it for energy metabolism. However, some glucose 1-phosphate derived from glycogen by glycogenolysis is directed into the glycolytic pathway and completely oxidized in the tricarboxylic acid cycle, ultimately yielding some ATP for use by the liver. Some of the triose phosphate produced in glycolysis, however, is diverted for synthesis of L- α -glycerol phosphate, which is used in the production of triacylglycerols.

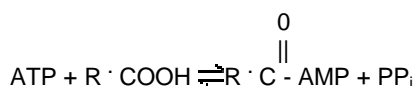
The liver, like other fatty acid--synthesizing tissues, including adipose tissue and mammary gland, has the enzymes of the pentose phosphate cycle (hexose monophosphate pathway), the oxidative branch of which provides NADPH for reductive synthesis of fatty acids. Although much experimental difficulty is encountered in evaluating the amount of glucose that goes through the oxidative arm of the pentose phosphate cycle as opposed to the glycolytic pathway (and indeed considerable effort has been expended for that purpose), such an estimation for the liver may not have great physiological significance. Normally, the liver does not have a large rate of glycolysis from glucose resulting in production of pyruvate or lactate; also, the oxidative branch of the pentose phosphate cycle is virtually suppressed. However, under conditions of lipogenesis, which cause NADPH to be drawn off for fatty acid synthesis, the pentose phosphate cycle would become active. In any case, glucose oxidation by the pentose phosphate cycle does not result in formation of ATP.

One can obtain some measure of the dependence of the liver on oxygen by examining an energy-requiring biosynthetic function carried out by hepatocytes: production of albumin. In humans, albumin is manufactured exclusively by hepatocytes in an amount of approximately 12 g or 0.18 mmol/day (30). Since a molecule of albumin contains 585 amino acid residues, about that many peptide bonds are formed. The synthesis of each peptide bond requires the input of about five high-energy pyrophosphate bonds; accordingly, the total daily synthesis of albumin requires about 0.526 moles ATP. The complete oxidation of a molecule of glucose to carbon dioxide and water can produce up to 38 molecules of ATP; the complete oxidation of a molecule of palmitic acid yields 129 molecules of ATP. To make 0.18 mmol albumin, the liver must oxidize 13.8 mmol glucose (2.48 g) or 4.0 mmol palmitic acid (1.02 g). The oxidation of that amount of glucose entails the consumption of about 82 mmol or 1.9 liters oxygen; the corresponding figure for palmitic acid is 85 mmol or 2.0 liters oxygen. These values are simply for polypeptide synthesis and do not include the additional energy needed for processing and transport of albumin.

HIGH-ENERGY COMPOUNDS USED BY THE LIVER

Ultimately, all the energy used by animals arises from oxidation of substrates. The principal form in which energy is preserved is the pyrophosphate bond of ATP; a minor fraction of the energy is conserved in GTP formed in a substrate level phosphorylation in the tricarboxylic acid cycle when α -ketoglutarate is oxidatively decarboxylated to succinate. A second generation of high-energy bonds can be formed by the transfer of the terminal phosphate group of ATP to a suitable acceptor molecule such as a nucleoside diphosphate; in that case, little free energy is lost, and new nucleoside triphosphates are formed that can be used in specific syntheses. In that category of reactions, for example, UDP is converted to UTP by reaction with ATP; the UTP so formed can be used in synthesis of nucleic acids or uridine diphosphateglucose (UDP-glucose). Similarly, CDP can be phosphorylated by ATP to form CTP; and the latter can be used in synthesis of nucleic acids or cytidine diphosphate choline (CDP-choline).

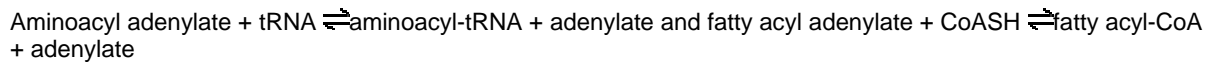
A third generation of high-energy phosphoanhydride bonds is formed when a nucleoside triphosphate transfers its nucleotidyl moiety to the acyl group of an organic acid to form an acylphosphoanhydride with little change of free energy; another product of the reaction is inorganic pyrophosphate. The nucleoside triphosphate used most frequently is ATP, although reactions are known also with CTP, UTP, and GTP. The general reaction with ATP is



This reaction can be driven to the right by hydrolysis of the high-energy inorganic pyrophosphate producing inorganic phosphate; an inorganic pyrophosphatase catalyzes this cleavage.

The formation of acylphosphoanhydrides occurs in the activation of amino acids to form aminoacyl adenylates used in protein biosynthesis, and in formation of fatty acyl adenylates in prelude to oxidation of fatty acids. The activated acyl group can then be transferred to a specific intermediate in the process. Thus, in protein biosynthesis, the activated aminoacyl group is transferred to the terminal adenylate residue of a specific tRNA molecule; in fatty acid oxidation, the fatty acyl moiety is transferred to the sulfur atom of CoASH. Both kinds of

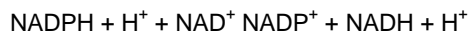
reactions occur with little change in free energy, thus keeping the newly formed acyl derivatives in an activated state:



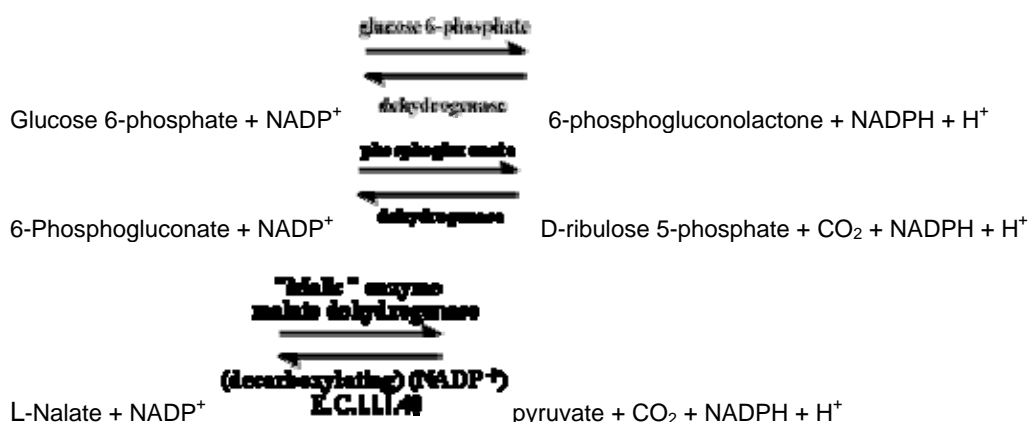
Except for one substrate-level phosphorylation step and one internal oxidation-reduction step in glycolysis, and one substrate-level phosphorylation reaction in the tricarboxylic acid cycle that results in formation of GTP, the reactions leading to synthesis of high-energy pyrophosphate bonds (ATP) occur in conjunction with the mitochondrial respiratory chain. That requires oxidation of a substrate to occur with reduction of either NAD^+ or FAD to form NADH and FADH_2 , respectively, and reoxidation of the coenzyme through the electron transport chain in the presence of molecular oxygen, ADP, and inorganic phosphate. NADPH, which is formed in a reaction utilizing NADP^+ , cannot be reoxidized directly by the mitochondrial respiratory chain and, therefore, cannot be coupled with production of ATP. Table 1 shows the steps in glycolysis, the tricarboxylic acid cycle including oxidation of pyruvate, and fatty acid oxidation that lead to production of ATP and GTP.

In the liver, the generation of ATP resulting from the reoxidation of NADH formed in the glutamate dehydrogenase reaction may, under certain circumstances, be of quantitative significance. For instance, when the amount of protein in the diet is in excess of that needed to provide amino acids for protein and nucleic acid synthesis, many of the amino acids can be transaminated with α -ketoglutarate to form glutamate and their corresponding α -keto acids; the glutamate can be oxidatively deaminated to ammonia and α -ketoglutarate, while NAD^+ is simultaneously reduced to NADH. NADH can be reoxidized in the presence of molecular oxygen, ADP, and inorganic phosphate with production of three molecules of ATP by oxidative phosphorylation. This amount of ATP is almost equivalent to the energy required for synthesis of urea from ammonia formed in the deamination reaction. However, should the glutamate dehydrogenase operate with NADP^+ instead of with NAD^+ , as can occur (33,34), no ATP would be generated with reoxidation of the coenzyme. Instead, the energy made available by oxidation of substrate is preserved, as described below.

When a substrate is oxidized by a dehydrogenase that uses NADP^+ , the NADPH formed cannot be directly reoxidized through the electron transport chain. The energy remains resident in the C---H bond formed when the pyridinium ring of the coenzyme is reduced. In certain highly specific reactions, constituting direct or indirect transhydrogenation systems, the following overall reaction occurs:



NADH then can be sent through the respiratory chain to produce three molecules of ATP. Thus the C---H bond in NADPH has sufficient energy to make three ATP pyrophosphate bonds biologically. In fact, most of the NADPH produced in the liver does not undergo transhydrogenation with NAD^+ but is employed directly for certain reductive syntheses that bypass the use of ATP. Two such reductive steps requiring the input of energy and of H atoms occur in synthesis of fatty acids from acetyl-CoA, a major activity of the liver. These reactions are concerned with reduction of a carbonyl to an alcohol group, and reduction of a carbon---carbon double bond to a carbon---carbon single bond (see Fig. 7). The NADPH used for these syntheses arises primarily from three oxidative reactions, as shown below:



GENERAL USES OF ENERGY IN THE LIVER

Approximately one-half the energy available to the liver is utilized in various kinds of transport (especially Na^+) and secretory functions (30,35); ions and substrates are carried into and out of cells, end products are removed, bile is formulated and secreted, and osmotic equilibrium is maintained by movement of water between extracellular and intracellular compartments. Some substances move across barriers by passive or facilitated diffusion without expenditure of energy, as is probably the case for fatty acids, glucose, and urea. For purposes of export, however, triacylglycerols, phospholipids, and cholesterol esters are incorporated into specific lipoproteins whose synthesis requires investment of energy.

Another utilization of energy is in the storage of fuels. Formation of glycogen, triacylglycerols, and phospholipids requires energy, as does the intrahepatic synthesis of glucose by gluconeogenesis. The syntheses of proteins and nucleic acids, urea, and a multitude of other substances are all highly energy-requiring reactions. Indeed, the biosynthetic activity of the liver reads like a litany of biochemistry. Biotransformation reactions of metabolites and drugs, such as the formation of bilirubin glucuronides, are energy consuming.

The following discussion mainly concerns reactions that occur in the parenchymal cells of the liver. Kupffer, endothelial, and other cells in the liver are more specialized in function than is the hepatocyte and generate much of their energy (as ATP and NADPH) by the metabolism of glucose. Hepatocytes in themselves constitute a metabolically heterogeneous population (36). In another section of this chapter we discuss hepatocyte heterogeneity and metabolic zonation.

UTILIZATION OF SUGARS BY THE LIVER

The carbohydrates presented to the liver after digestion are principally glucose, fructose, and, especially in infancy and childhood, galactose.

The uptake of glucose by the liver has recently been investigated extensively (37). In that regard, consideration has especially been given to the function of nervous control (38), hormones and glucose *per se* (39), and other factors including a portal signal (40).

Glucose and other hexoses must be transported into and out of hepatocytes. The outward transport is critical for the support of glucose concentrations in the blood that are necessary for the nutrition of organs other than the liver. Hepatocyte membranes are equipped with hexose transporters (41---45) that facilitate the movement of the sugars into and out of the cells. The main transporter of glucose in the hepatocyte is called GLUT-2 (43---45). It is not present in Kupffer cells. Its expression is limited to sinusoidal membranes, and it is not present in the apical, bile canalicular membranes. Unlike GLUT-4 present in muscle and adipose tissue cells, GLUT-2 of hepatocytes is not translocated to intracellular membranous vesicles, but remains associated with the plasma membrane. One may recall that GLUT-4 is translocated from plasma membranes to intracellular membranes in response to certain signals, and translocated back to the plasma membrane in response to insulin. These facts are consistent with the insensitivity of the GLUT-2 transporter to insulin, and the sensitivity of GLUT-4 to insulin.

The properties of GLUT-2 are appropriate for the function of hepatocytes in the handling of glucose. The k_m for glucose being transported into hepatocytes is 15 to 20 mM. (This can be compared to a k_m of 1 to 3 mM glucose for the GLUT-1 transporter of the erythrocyte.) The transporter in hepatocytes is symmetrical, i.e., the k_m values for glucose entry and glucose exit are both about 20 mM. GLUT-2 of the hepatocyte, with its high k_m for glucose, has low affinity for the sugar. The high k_m allows the uptake of glucose by hepatocytes to increase in proportion to its rise in concentration after a carbohydrate meal.

GLUT-2 occurs in isolated hepatocytes, basolateral membranes of intestinal cells, and in pancreatic islet cells. One may assume that it is the dominant glucose transporter in these cells. It is inhibited by relatively high concentrations of cytochalasin B as compared with GLUT-1.

Although the structural basis for the kinetic and affinity properties of GLUT-2 are not known, some workers have suggested that it may be related to a unique feature of GLUT-2 among transporters, that is, it contains a segment of glycosylated exoplasmic loop between the first and second transmembrane domains of the transporter.

After an individual eats a meal rich in carbohydrate, the concentration of glucose in the portal blood is greater than the normal concentration of approximately 5 mM. Glucose is taken into liver cells where it undergoes glycolysis and/or may be converted to and stored as glycogen. Insulin favors the storage of glucose by increasing the level of glucokinase and the process of glycogen synthesis. In another locus, insulin decreases the level of phosphoenolpyruvate carboxykinase (PEPCK). The corresponding counter-regulatory hormones are glucagon, epinephrine, and glucocorticoids; these promote an increase in the level of cAMP, an effector that promotes the transcription of the PEPCK gene. Thus the movement of glucose into and out of hepatocytes is controlled by

hormonal regulation of enzymes involved in glycolysis, glycogen synthesis, and gluconeogenesis, and not by hormonal regulation of the glucose transporter (GLUT-2) *per se*.

GLUT-1 (the glucose transporter characteristic of the erythrocyte) is also expressed in liver but only to the extent of about 1% to 2% of GLUT-2. After an individual fasts, the hepatic GLUT-1 increases severalfold. GLUT-1 seems to occur in a group of hepatocytes located around the central vein; after an animal fasts, the number of cells containing GLUT-1 in this region seems to increase.

Experiments have been performed to determine whether hyperglycemia induced in several different ways can influence the level of mRNA for GLUT-2 and/or the level of protein for that transporter. Two forms of non-insulin--dependent diabetes were studied, that produced in rats with streptozocin and that occurring in Zucker rats; the results showed that hyperglycemia was not accompanied by increases in the levels of mRNA and protein for GLUT-2. In other experiments, however, fasted rats exhibited a significant decrease in GLUT-2 mRNA, but no significant change in GLUT-2 protein. That surprising apparent discrepancy may point to a complex regulation of expression in GLUT-2 in liver as has been described for glucokinase.

Finally, as in the case of glucokinase, GLUT-2 is also expressed in the β -cells of the pancreatic islets and in epithelial cells of intestinal and kidney cells. Both glucokinase and GLUT-2 operate at relatively high k_m values, and it would seem that the GLUT-2 transporter makes possible the achievement of appropriate concentrations of glucose in the cell so that glucokinase can function efficiently.

As studied in the rat (46), the glucose transporter provides glucose to hepatocytes at a rate approximately 600 times greater than the rate of phosphorylation by hexokinase and approximately 100 times greater than the rate of phosphorylation by glucokinase, so that metabolism of the sugar is not limited by transport-in. Nor is transport of glucose out of the cells limiting for supply of blood glucose by hepatocytes, since the capacity for dephosphorylating glucose by action of glucose-6-phosphatase is about one-third of the rate of outflow of the sugar (42,47).

In preparation for its metabolism, each sugar may be phosphorylated by a kinase. In hepatocytes the principal phosphorylating enzyme for glucose, hexokinase IV, is one of the four known mammalian hexokinases (48--54); this is usually referred to as glucokinase although it also can phosphorylate mannose and 2-deoxyglucose. Glucokinase operates at relatively high concentrations of glucose, having a k_m for that substrate of about 10-2 M; other hexokinases exhibit a k_m for glucose of about 10-4 M. Thus the k_m for glucokinase is greater than a normal plasma concentration of glucose and is in the range of concentrations of glucose that could occur in the portal blood. Glucokinase exhibits a sigmoidal curve for saturation with glucose. That is considered to arise from the existence of the enzyme in two conformational states, one of which has high affinity for glucose and the other low affinity. The enzyme, which is not active after a catalytic round, can return rapidly from the high-affinity form to the low-affinity state.

Unlike other hexokinases, glucokinase is not inhibited by its product, glucose 6-phosphate, except at relatively high concentrations. It is inhibited reversibly by long-chain acyl-CoAs, although the physiological significance of such inhibition, if in fact it occurs *in vivo*, is not apparent. Glucokinase is also inhibited by a regulatory protein of about 60 kDa molecular mass (55), but only when fructose 6-phosphate is present. Fructose 6-phosphate alone does not inhibit. Experiments have been performed showing that, in the presence of fructose 1-phosphate, the regulatory protein forms a complex with glucokinase. The regulatory protein alone can bind fructose 1-phosphate or sorbitol 6-phosphate with 1:1 stoichiometry. In the model proposed (55), one conformational form of the regulatory protein, R, binds to glucokinase to inhibit it; a second conformational form, R', cannot bind to glucokinase and thus does not inhibit the enzyme. Fructose 6-phosphate can bind to the R conformer and reinforce inhibition of glucokinase. On the other hand, fructose 1-phosphate traps the regulatory protein in its R' conformation and antagonizes the effect produced by fructose 6-phosphate. Physiologically, the hepatocyte contains a relatively high concentration of fructose 6-phosphate and almost no detectable amount of fructose 1-phosphate; thus the regulatory protein is able to inhibit glucokinase. Under those conditions in the hepatocyte the apparent affinity of glucokinase for glucose is diminished when compared to isolated, pure glucokinase. There is evidence that such regulation indeed occurs *in vivo*.

The regulatory protein binds at a site distant from the catalytic site. The stimulation of glucokinase activity by fructose may be due to formation of fructose 1-phosphate that antagonizes inhibition by the regulatory protein. Fructose thus could be a nutritional signal stimulating uptake of glucose in the liver (55).

The gene for glucokinase (56,57) is expressed in pancreatic β -cells as well as in hepatocytes. The expression of glucokinase in hepatocytes is stimulated by insulin (48) and repressed by cAMP. Glucokinase activity in β -cells of the pancreatic islets is increased by glucose. Current evidence points to the occurrence of two different transcription control sites in the glucokinase gene, one operating in the liver and the other in β -cells of the

pancreas. In the liver, only one isoform of glucokinase arises from its gene, but in the pancreatic β -cells two isoforms arise from the same gene.

With respect to its function in the liver, glucokinase could possibly have evolved in relation to its importance for the synthesis of glycogen. However, a proposal has been made that glucokinase also may serve as a sensor in the liver as it does in the endocrine pancreas (58).

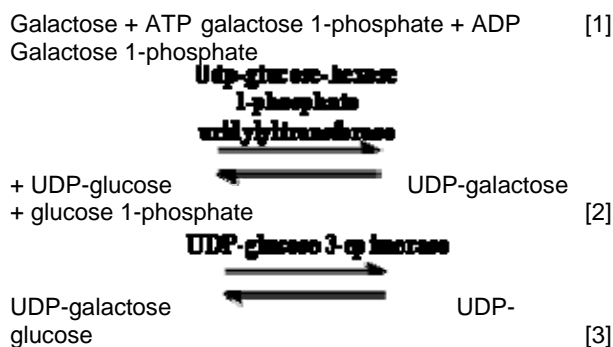
Fructose is phosphorylated in hepatocytes by a fructokinase or ketohexokinase, with participation of ATP or GTP (59--65). The product is fructose 1-phosphate. Fructokinase is highly active in liver. Although fructose can ultimately form glycogen by conversion to glucose 6-phosphate, fructose 1-phosphate can traverse the glycolytic pathway more readily than glucose. Glycolysis results in the production of pyruvate, which can then be oxidized to acetyl-CoA, a precursor of fatty acid synthesis, and a side reaction of glycolysis results in formation of L- α -glycerol phosphate, which is required in the synthesis of triacylglycerols. Thus fructose has greater flux through glycolysis than glucose and is a better substrate for lipogenesis in the liver.

METABOLISM OF GALACTOSE IN THE LIVER

Galactose occurs in the diet primarily in the form of the disaccharide lactose. Human milk contains about 7% lactose and bovine milk contains about 5%. Galactose consumed in excess of the need of the organism for biosynthesis of glycoproteins and galactolipids is metabolized chiefly in the liver. It can ultimately be incorporated as glucosyl units in glycogen or be converted to glucose 6-phosphate and enter the glycolytic pathway for oxidation and provision of energy.

Pyruvate formed from galactose in that manner obviously can be oxidized further to produce acetyl-CoA, with the options open to acetyl-CoA for additional production of energy through oxidation in the tricarboxylic acid cycle or for biosynthesis of fatty acids.

The first reaction of galactose in the liver is with ATP to form galactose 1-phosphate; the governing enzyme is a specific galactokinase. The metabolism of galactose in human liver follows the Leloir pathway (66,67):



Depending on the metabolic state of the liver, glucose 1-phosphate formed in the transferase reaction can be converted to glucose 6-phosphate and enter the glycolytic pathway for production of energy. Also, UDP-glucose arising in the epimerase reaction may be directed toward glycogenesis.

With respect to the epimerase, its action requires NAD^+ as a coenzyme. NAD^+ removes a hydrogen atom from C-4 of the UDP hexose as well as one from the -OH on C-4; an intermediate UDP-ketohexose forms. The NADH formed in the reaction remains bound to the enzyme, which then transfers the hydrogen atom to a molecule of UDP ketohexose in such a way as to produce a UDP-hexose with the -OH at C-4 in an opposite configuration. In the human, if ethanol has been consumed with galactose, its oxidation by alcohol dehydrogenase in the liver significantly increases the amount of coenzyme in the form of NADH and consequently increases the ratio of NADH to NAD^+ . Such increase can inhibit the epimerase and decrease conversion of UDP-galactose to UDP-glucose. The overall effect is diminished clearance of galactose by the liver and from blood (68). In that situation, decreased tolerance to galactose has a metabolic basis without overt pathology.

METABOLISM OF FRUCTOSE (AND GLYCERALDEHYDE)

Unlike the phosphorylation of fructose in extrahepatic tissues, which occurs through the agency of a general hexokinase and results in formation of fructose 6-phosphate, phosphorylation in the liver occurs with a specific fructokinase, called ketohexokinase; the product is fructose 1-phosphate (59---65,69). Accordingly, fructose in the liver cannot directly enter glycolysis to any significant degree. Instead, the liver is equipped with a special aldolase, called fructose 1-phosphate aldolase or aldolase B to distinguish it from the aldolase A that acts on fructose 1,6-bisphosphate in the glycolytic pathway (63,70---74). Although aldolase B can also effectively catalyze the reversible reaction between fructose 1,6-bisphosphate and the two triose phosphates (glyceraldehyde 3-phosphate and dihydroxyacetone phosphate), aldolase A acts only weakly on the reversible conversion of fructose 1-phosphate to dihydroxyacetone phosphate and glyceraldehyde.

Glyceraldehyde formed by aldolase B can be phosphorylated by a specific triokinase (glyceraldehyde kinase) to produce glyceraldehyde 3-phosphate (63,71,75); the enzyme can also utilize GTP (76). In the metabolism of fructose in the liver, the aldolases form fructose 1,6-bisphosphate and eliminate the need for participation of 6-phosphofructokinase. The importance of that circumstance is that the early steps of fructose utilization in the liver are free from many controls. One may recall that the flux of glucose through glycolysis is regulated largely at the level of 6-phosphofructokinase (also designated later as 6PF-1K; see "Abbreviations and Designations" at end of chapter); the activity of that enzyme is modulated by many allosteric effectors (ATP, ADP, AMP, P_i , citrate) and by glucagon and epinephrine (77---79). However, the most potent effector has been found to be fructose 2,6-bisphosphate (80---90), whose synthesis is diminished by the action of glucagon (87---91). Since fructose 2,6-bisphosphate stimulates the 6-phosphofructokinase reaction and, therefore, the flux of glucose through glycolysis, the action of glucagon is to diminish that flux. The great significance of this for the metabolism of fructose is that the bypass of 6-phosphofructokinase allows fructose to go through glycolysis with fewer restraints than those imposed on glucose. The result is more ready formation of pyruvate and, accordingly, of acetyl-CoA for synthesis of fatty acids. It also allows more ready formation of a byproduct of glycolysis, namely, the L- α -glycerol phosphate that is used in esterification of fatty acids to form triacylglycerols. Thus, it is clear why lipogenesis in the liver is greater from fructose than from glucose.

If the only action of glucagon were at the level of the phosphofructokinase reaction, the therapeutic use of fructose would seem to have justification in diabetic persons with a glucagon---insulin imbalance. However, glucagon also may indirectly decrease the flux of pyruvate through the pyruvate dehydrogenase system, where insulin is important as a countervailing hormone. In any case, the use of intravenous alimentation with fructose has important side effects that make its use questionable and perhaps dangerous. The side effects can be observed in normal animals and humans taking a large fructose meal but are exaggerated in patients with hereditary fructose intolerance in whom liver aldolase B is lacking. Accumulation of fructose 1-phosphate occurs in both cases (63,64,69,92,93). The phenomenon can be duplicated and studied in isolated livers of rats perfused with solutions containing fructose; in this case, accumulation of fructose 1-phosphate occurs coordinately with depletion of ATP and P_i (69,72,93,94). Because fructokinase can utilize GTP as well as ATP, GTP in the liver also may be depleted after administration of a large amount of fructose (65). Of course, in normal humans (or rats) the effect is not due to lack of aldolase B, as in hereditary fructose intolerance, nor to an imbalance between ketohexokinase activity and aldolase B activity, since these are normally of about the same order of magnitude. The initial depletion of ATP is caused by rapid phosphorylation of fructose with ATP catalyzed by ketohexokinase. Ultimately that leads to sequestration of P_i into fructose 1-phosphate. The initial depletion of ATP and P_i constitutes removal of two important restraints on the catabolism of adenine nucleotides, since both inhibit 5'-nucleotidase and adenosine deaminase (69,72,94). Accordingly, degradation of adenine nucleotides is enhanced, resulting in diminution of the total pool of adenine nucleotides. For instance, the perfused rat livers referred to above show a decrease in that pool to a level of one-third to one-quarter of normal. The metabolic consequences of that kind of decrease coupled with diminution of the concentration of P_i can be very severe. Thus, under such conditions of diminished adenine nucleotides, the liver behaves almost as though it were severely hypoxic, and in fact becomes a lactate-producing organ (95). In the human, it could cause lactic acidosis. The mechanisms for those effects are not fully understood, but the dangers are sufficient to discourage the use of parenteral fructose solutions for nutritional purposes (64,69,95---97).

GLYCOLYSIS

The glycolytic pathway, also known as the Embden-Meyerhof-Parnas pathway, is the only mechanism by which glucose (or sugars interconvertible with it) can be oxidized anaerobically with production of ATP. Thus when a tissue such as liver, which normally operates under highly aerobic conditions, is rendered hypoxic, production of ATP by oxidative phosphorylation is greatly diminished and glycolysis is greatly increased. Glycolysis can operate as a cycle under hypoxic or anaerobic conditions when pyruvate is transformed to lactate by lactate dehydrogenase; NADH is thereby reoxidized to NAD^+ to allow the critical step of oxidation of glyceraldehyde 3-phosphate to continue. The total capacity for glycolysis can be estimated by measurement of lactate production in the absence of oxygen when suspensions of cells, slices of tissues, or whole perfused organs are permitted to metabolize glucose (98---103). The potential for glycolysis of the liver of a fed adult rat is about 150 mmol/g as determined from lactate production of isolated perfused livers under anaerobic conditions (104). However, no

uncomplicated estimate of glycolytic potential of human liver has been made. The value is probably in the range of production of 100 to 200 g lactate per day (95).

Under normal aerobic conditions, the liver primarily uses fatty acids as substrates for oxidation, and the glycolytic rate is low. Glycolysis occurs mainly from stored glycogen; little proceeds directly from glucose *per se*. Indeed, in the liver the extent of phosphorylation of glucose with formation of glucose 6-phosphate is not very great (105) and could be limiting for glycolysis and for the other processes dependent on that phosphorylated intermediate, including glycogen formation and the pentose phosphate cycle. Higher rates of glycolysis are associated with lipogenesis from carbohydrate. The carbon atoms of the sugar provide both acetyl-CoA for synthesis of fatty acids and L- α -glycerol phosphate for their esterification. Glycolysis, directed toward lipogenesis, occurs more readily with fructose or sorbitol than with glucose.

Flux of glucosyl units through the glycolytic pathway in the first instance depends on controls imposed on glycogen metabolism. For example, high concentrations of glucose entering hepatocytes activate glycogen synthase and diminish the activity of phosphorylase, thus favoring glycogenesis over glycogenolysis (106) and resulting in less substrate available for glycolysis. Normal levels of glucose are compatible with glycogenolysis, with much of the resulting glucose 6-phosphate being hydrolyzed to free glucose by glucose 6-phosphatase and made available as blood glucose. However, control at the level of glycogen metabolism is coordinated with controls on the irreversible reactions in the glycolytic pathway.

Starting with free glucose, the pathway has three physiologically irreversible reactions catalyzed by, respectively, glucokinase or hexokinase, 6-phosphofructokinase, and pyruvate kinase. Hexokinase, but not glucokinase, is inhibited by glucose 6-phosphate in physiological concentrations. 6-Phosphofructokinase is inhibited by ATP and, when citrate is present with ATP, more strongly than by ATP alone. Inhibition of 6-phosphofructokinase by ATP is relieved by P_i , AMP, ADP, fructose 6-phosphate, or fructose 1,6-bisphosphate (77---79). The most significant activation of 6-phosphofructokinase, however, occurs in the presence of another metabolite, fructose 2,6-bisphosphate (80---90, 107---110). On a micromolar basis, this compound is about 1000 times more active than fructose 1,6-bisphosphate (86); it acts by increasing the affinity of fructose 6-phosphate for 6-phosphofructokinase, thereby relieving inhibition by ATP. Fructose 2,6-bisphosphate has a second effect, namely, the inhibition of fructose 1,6-bisphosphatase, and accordingly represses gluconeogenesis (87---90).

The enzyme 6PF-2K/F2,6-P₂ase is bifunctional, that is, it has one of two separate catalytic activities depending on whether or not it is phosphorylated. The enzyme of the liver can be phosphorylated by a cyclic AMP-dependent protein kinase. In the phosphorylated state it catalyzes the hydrolysis of fructose 2,6-bisphosphate to fructose 6-phosphate and inorganic phosphate (Fru_{2,6}-P₂ase activity) and has minimal activity as a specific kinase for the phosphorylation with ATP of fructose 6-phosphate to fructose 2,6-bisphosphate (6PF-2K activity). When dephosphorylated by a phosphatase, the enzyme again exhibits 6PF-2K activity and little Fru_{2,6}-P₂ase activity. Thus in its phosphorylated state, the enzyme causes a decrease in the amount of the powerful effector, fructose 2,6-bisphosphate, thereby decreasing the flux of glucose through glycolysis and stimulating the gluconeogenic pathway. In a reciprocal way, the enzyme in its unphosphorylated state promotes an increase in the amount of fructose 2,6-bisphosphate, thereby promoting the flux of glucose through glycolysis and inhibiting the gluconeogenic pathway. As discussed under "Gluconeogenesis" below, glucagon, acting through cyclic AMP, promotes the phosphorylation of the enzyme and thereby stimulates gluconeogenesis (87---91, 108, 109).

The third irreversible reaction in glycolysis is catalyzed by pyruvate kinase. That enzyme can be phosphorylated by a cyclic AMP-dependent protein kinase which results in its inactivation; dephosphorylation by a phosphatase results in reactivation (111, 112). Pyruvate kinase is also activated by fructose 1,6-bisphosphate; it is inhibited by ATP and by some amino acids, notably alanine, which is an important precursor in gluconeogenesis (113). In relation to the activation of pyruvate kinase by fructose 1,6-bisphosphate, one can infer that a decrease in 6PF-1K activity, resulting from a decrease in levels of fructose 2,6-bisphosphate, would diminish the amount of fructose 1,6-bisphosphate and result in decreased feed-forward activation of pyruvate kinase. Thus the two substrate cycles operating in the direction of glycolysis are coordinated.

In the liver, glycolysis is closely related to glycogen deposition (synthesis and glycogenolysis), lipogenesis, and gluconeogenesis. Normally, glycolysis and gluconeogenesis occur simultaneously; however, as discussed later, the two processes may occur separately in hepatocytes located, respectively, in perivenous and periportal zones. Each of the irreversible steps in glycolysis is matched by an irreversible step in gluconeogenesis. Thus interconversion of pyruvate and phosphoenolpyruvate occurs because the action of pyruvate kinase in glycolysis is countered by the combined actions of pyruvate carboxylase and phosphoenolpyruvate carboxykinase in gluconeogenesis. Interconversion of fructose 6-phosphate and fructose 1,6-bisphosphate is a result of the action of 6-phosphofructokinase in glycolysis countered by the action of fructose 1,6-bisphosphatase in gluconeogenesis. Interconversion of glucose and glucose 6-phosphate occurs because the action of glucokinase or hexokinase is opposed by the action of glucose 6-phosphatase in gluconeogenesis. At each of those levels, "futile cycling" of a substrate could occur, the net balance of which would be the hydrolysis of ATP with production of ADP and P_i (106, 114). The direction in which a substrate will go at each of these levels is

determined by its concentration and by controls exerted on the enzymes by allosteric effectors. The relatively small amount of energy expended in the cycling of a substrate is not a great price to pay for the control of metabolism at that point. In the case of phosphoenolpyruvate, normal cycling can be pushed in the direction of gluconeogenesis by the action of glucagon, which, through a cyclic AMP---dependent protein kinase, causes inactivation of pyruvate kinase; that results in an increase in the amount of phosphoenolpyruvate and stimulation of the gluconeogenic pathway.

No significant substrate cycling has been found in livers of mice or rats at the level of interconversion of fructose 6-phosphate and fructose 1,6-bisphosphate. However, such cycling does occur in hepatocytes isolated from rat livers. Glucagon has a profound effect on this cycle, strongly favoring the gluconeogenic pathway (115). Since glucagon interferes with the synthesis of the powerful effector, fructose 2,6-bisphosphate, the flux of fructose 6-phosphate through the 6-phosphofructokinase reaction is slowed considerably by glucagon. Moreover, fructose 2,6-bisphosphate inhibits fructose 1,6-bisphosphatase, so that the action of glucagon removes a restraint on the enzyme and increases the concentration of fructose 6-phosphate (87---90,116---123), which also favors gluconeogenesis.

Recycling between glucose and glucose 6-phosphate in the liver is catalyzed by glucokinase and glucose 6-phosphatase; since the former is a "soluble cytosolic" enzyme and the latter is associated with the endoplasmic reticulum (ER), cycling in this case must include the movement of glucose 6-phosphate into the ER and movement of glucose out. Both enzymes are characterized by relatively high k_m values for their substrates; in the short term, both are controlled principally by the concentration of substrate. This system offers an excellent example of the effect of substrate level per se on flux through the glycolytic pathway. Thus, it has been determined that no net flux of the two metabolites occurs when the blood level of glucose is about 100 mg/100 ml (5.7 mmol/liter) because the activity of glucokinase becomes equal to the activity of glucose 6-phosphatase (106). An increase of the concentration of either substrate has no influence on the net flux, since the product of the first reaction is returned by the reverse reaction. In the hepatocyte, the presence of systems for metabolism of glycogen, which can be controlled by factors other than substrate concentrations, produces a coordinate control on the level of glucose 6-phosphate. Thus an increased concentration of glucose gives a transient increase in the level of glucose 6-phosphate, but since glucose overall also depresses phosphorylase and stimulates glycogen synthase, glucose 6-phosphate is drawn off for formation of glycogen, and its level undergoes a secondary decrease. With the decrease of glucose 6-phosphate, the activity of glucose 6-phosphatase is decreased. A large difference occurs in the activity of glucokinase as opposed to the phosphatase. This permits active uptake of glucose for glycogen synthesis. When glycogenolysis is active, as caused by glucagon, glucose 6-phosphate concentration increases considerably and causes increased activity of glucose 6-phosphatase. Aided by GLUT-2, the glucose that forms diffuses rapidly out of the hepatocytes into the circulation for distribution to other tissues. The change in concentration of glucose is small. What then is the advantage of recycling of these substrates? Hers (106) states that it allows large changes in glucose uptake and output to be regulated only by substrate concentration; however, it requires cooperation of the phosphorylase-glycogen synthase system.

GLUCONEOGENESIS

The major similarities and differences between the pathways for gluconeogenesis and glycolysis, and the interactions of the two, have been considered in the discussion of glycolysis.

Gluconeogenesis, the production of glucose principally from amino acids and lactate, is carried out solely in liver and kidney cortex; in liver, it apparently occurs mainly in hepatocytes located in the periportal zone. Although the rates of gluconeogenesis are similar in the two organs, total production by the liver is probably about nine times as great because of its relatively larger size. However, because gluconeogenesis in the kidney serves special functions in relation to maintenance of ammonia production and acid-base balance, and because conditions occur in which gluconeogenesis increases in that organ, the significance of the renal process cannot be discounted. The discussion here, however, is limited to the liver.

Major extrahepatic tissues utilize glucose obligatorily (blood cells, renal medulla, and retina); others use it preferentially (brain). However, only liver and kidney make glucose in significant amounts. In the postabsorptive period, or during periods of fast, the extrahepatic tissues send precursors to the liver via the blood to be converted to glucose, which is distributed to the tissues to be used as fuel. Two interorgan cycles of that kind have been described: the lactic acid or Cori cycle (124) and the glucose-alanine cycle (125,126). By the process of gluconeogenesis, liver can produce at least 240 g glucose per day (95), which is about twice the amount consumed by nervous tissue and erythrocytes in 1 day of fasting (127). In most cases, it is not the gluconeogenic capacity of the liver that is limiting, but the supply of precursors and perhaps of ATP. A major fraction of amino acid precursors for gluconeogenesis during fasting or starvation arises by degradation of functional muscle protein and protein in visceral organs, including the liver itself. The process of gluconeogenesis, if not regulated at the precursor level, becomes tissue wasting with dire consequences. The degradation of muscle protein in an extended fast declines to a relatively constant level; it primarily is spared not by provision of a different pool of

precursors for glucose synthesis but by replacing the use of glucose in nervous tissue and in skeletal muscle by acetoacetate (128---131). As discussed later, the production of acetoacetate, a fuel that spares conversion of protein to glucose, also occurs in the liver. Other aspects of regulation of glucose production by glycogenolysis and gluconeogenesis have been considered (132,133).

The obligate users of glucose, such as erythrocytes and renal medulla, have high rates of glycolysis and produce considerable lactate. Thus, the liver has available from blood cells a relatively constant but limited amount of substrate for gluconeogenesis. To this is added a more variable supply of precursors, including gluconeogenic amino acids, glycerol from lipolysis in the adipose tissue, and propionyl-CoA from oxidation in hepatocytes of fatty acids with an odd number of carbon atoms. A fatty acid with an odd number of carbon atoms is oxidized by β -oxidation, in which, starting from the carboxyl terminus, two carbon atoms at a time are removed sequentially as acetyl-CoA. At the other end of the chain, β -oxidation ceases with production of a three-carbon product, propionyl-CoA. Propionyl-CoA can be converted to methylmalonyl-CoA, which, by enzyme-catalyzed rearrangement to succinyl-CoA, is able to enter the tricarboxylic acid cycle.

Almost all amino acids, with the exception of leucine, can contribute some carbon atoms to the net synthesis of glucose in the liver; some also contribute carbon atoms to the synthesis of acetoacetate.

Although alanine coming from muscle is a major precursor of glucose, serine and other amino acids also are important in that regard. Amino acids are a principal source of glucose after liver glycogen has been nearly depleted by glycogenolysis.

Gluconeogenesis is both an energy-consuming and a reductive process. For two molecules of pyruvate to traverse the pathway for formation of a molecule of glucose requires investment of the energy of six pyrophosphate bonds. In addition, the H atoms of two molecules of NADH and two protons are required at the level of formation of glyceraldehyde 3-phosphate from 3-phospho-D-glyceroylphosphate (1,3-diphosphoglycerate). One may estimate that a 70-kg person uses about 17 kcal/day (about 5% of the approximate total of 340 kcal energy used by the liver in its functions) for conversion of lactate plus glycerol to glucose (30,35). The liver also uses about 48 kcal energy per day (14% of its total) for the degradation of amino acids; this includes the energy expended in synthesis of urea from the ammonia produced by oxidative deamination and the synthesis of both glucose and ketone bodies (30,35). Although all that energy utilization cannot be ascribed directly to the requirement of gluconeogenesis, it may reasonably be considered a necessary concomitant. Much of the energy required for gluconeogenesis is provided by oxidation of fatty acids in hepatocytes.

The control of gluconeogenesis is considered in some detail in the preceding section on "Glycolysis." Pyruvate carboxylase, at low concentrations of pyruvate, has an almost complete dependence on the presence of acetyl-CoA for activity (113,134). Phosphoenolpyruvate carboxykinase is controlled most effectively by changes at the transcriptional level in its rate of synthesis. Glucagon and glucocorticoids promote induction of the enzyme, whereas insulin represses its synthesis (57,135---138). In fed rats, gluconeogenesis in liver shows a circadian rhythm that corresponds to a rhythm in the activity of phosphoenolpyruvate carboxykinase (139). For lactate, pyruvate, glycerol, alanine, glutamate, aspartate, serine, and glycerol, the production of glucose was greater at 8 PM than at 8 AM. This was not true for glutamine.

Overall, gluconeogenesis is stimulated by glucagon and epinephrine and inhibited by insulin, as observed most dramatically in insulin-dependent diabetes mellitus, in which uninhibited gluconeogenesis contributes significantly to the hyperglycemia. Gluconeogenesis also can be affected indirectly by the level of activity of the pyruvate dehydrogenase reaction, which determines the flux of pyruvate away from glucose synthesis and toward oxidative decarboxylation to form acetyl-CoA. Insulin favors oxidative decarboxylation of pyruvate and, therefore, also indirectly tends to diminish gluconeogenesis. Many reviews of the hormonal regulation of gluconeogenesis have appeared (117,119---123,140---142).

Of considerable relevance to the subjects of gluconeogenesis, glycogen synthesis by the indirect pathway, the Cori cycle, and insulin resistance are recently described actions and possible functions of the pancreatic peptide, amylin (143---145).

Amylin, recommended by some to the status of a hormone (143), is a 37-amino acid peptide elaborated by pancreatic β -cells and secreted into the bloodstream together with insulin. While it is about 50% identical with calcitonin gene---related peptides, and can produce some of the physiological effects of those peptides (e.g., vasodilation), amylin also has very specific effects on carbohydrate metabolism. Thus in fed rats, in which gluconeogenesis is low, amylin causes a prolonged and pronounced rise in plasma lactate, but only a modest rise in plasma glucose. In fasted rats, in which gluconeogenesis is high, amylin promotes less of an increase in plasma lactate and a greater rise in plasma glucose. Experiments show that the rise in glucose owing to amylin

occurs from increased gluconeogenesis from lactate. Thus, the venerable Cori cycle receives new interest (as it does in the case of the indirect pathway of glycogen synthesis discussed later), and amylin can be considered to promote the hepatic recycling of lactate from the periphery into glucose. One may therefore infer that the two peptides from the β -cells of the pancreas act in a regulatory pairwise fashion: Amylin acts to stimulate glycogen production in the liver from lactate, and insulin operates on the other limb of the Cori cycle to stimulate the uptake of glucose by muscle and its conversion there to glycogen. Actions of amylin may also explain aspects of insulin resistance in non-insulin-dependent diabetic persons and the occurrence of hypoglycemic attacks in insulin-dependent diabetic individuals (143).

A recent interesting, speculative article proposes that abnormalities of lipid metabolism may be primary in certain aspects of diabetes (146). In that article the author considers the possibility that the hyperinsulinemia of early non-insulin-dependent diabetes is coincident with hyperamylinemia, since insulin and amylin are cosecreted. Amylin would cause an increase in plasma lactate (Cori cycle); and lactate, a better precursor than glucose for fatty acid synthesis, would indirectly promote the production of very-low-density lipoproteins (VLDL). There would follow an increased flux of triglycerides from liver to muscle (and adipose tissue) and, as proposed and elaborated on, an increase in insulin resistance and production of many of the metabolic disturbances occurring in diabetes.

Any disease in which carbohydrate metabolism in the liver is disturbed may have an effect on the capacity for gluconeogenesis and its regulation. Conditions in which oxygenation of the liver is diminished, as in congestive heart failure, affect almost all hepatocytes and may cause them to turn to glycolysis for production of energy. Consequently, the liver becomes a lactate-producing organ instead of one that directs lactate into gluconeogenesis. Lactic acidosis may ensue, but is not likely to happen in cirrhosis, in which sufficient numbers of hepatocytes may still function normally (95).

In humans, at least two inherited enzyme deficiencies have a major effect on the gluconeogenic pathway. These are type I glycogenosis [von Gierke's disease (147)], in which there is a deficiency of glucose 6-phosphatase, and hexose biphosphatase deficiency. Both are characterized by severe hypoglycemia and lactic acidosis. Patients with type I glycogenosis do not accumulate hepatic glucose 6-phosphate; Hers (106,147) has suggested that an unknown feedback mechanism inhibits gluconeogenesis. On the other hand, patients with diabetes mellitus may have gluconeogenesis in the face of hyperglycemia, indicating that a feedback control on the output of glucose from the liver is affected. Finally, one may note that dedifferentiated liver cells in many experimental hepatomas have a graded loss of gluconeogenic capacity (148).

THE PENTOSE PHOSPHATE CYCLE

This cycle, also called the hexose monophosphate pathway or shunt, consists of two branches that serve different functions in metabolism. Those branches may be designated A and B. The A arm, often called the oxidative pathway, catalyzes two successive dehydrogenations of glucose 6-phosphate, in which some of the energy of oxidation and one of the hydrogen atoms are retained in NADPH. Indeed, the primary function of the oxidative branch is production of NADPH for use in reductive syntheses, such as those involved in synthesis of fatty acids from acetyl-CoA. The final step in the oxidative branch also produces ribulose 5-phosphate, which becomes the starting substrate for the B branch. The latter, constituting an almost labyrinthal series of interconversions and rearrangements, has as its main function the production of ribose 5-phosphate for use in synthesis of nucleotides and nucleic acids. It also allows for production of glucose 6-phosphate that can be recycled into the A branch. Thus, the pentose phosphate cycle is not an "alternate" pathway for the oxidation of glucose. It cannot replace the functions of glycolysis, chief of which is the preservation of energy of oxidation in the form of pyrophosphate bonds of ATP; nor can glycolysis replace the functions of the pentose phosphate cycle as outlined.

Not all tissues contain the enzymatic equipment necessary for the pentose phosphate cycle. The enzymes of that cycle notably are absent or deficient in the skeletal muscle. The liver, however, has all the enzymes in sufficient amount so that under certain physiological conditions, significant metabolism of glucose can occur through the pentose phosphate cycle (149). Hepatocytes are the main locus of the cycle, but other hepatic cells contain some or all of the necessary elements.

Some investigators (150,151) have stated that the oxidative arm of the classical pentose phosphate pathway, active in adipose tissue, does not function in the liver; indeed they propose a separate pathway, termed L, in the liver. This conclusion has been in dispute. Others pursuing this matter (152,153) present evidence strongly supporting the contention that the metabolism of glucose by the pentose phosphate cycle proceeds by the classical pathway and not by the proposed L pathway.

Since the liver has the capacity to produce more ribose 5-phosphate than required for its own metabolic needs, it may export ribose derivatives including ribose nucleosides to other tissues (154). Such nonphosphorylated forms can cross liver cell membranes, appear in the blood, and then be extracted by various cells of the organism. Adenosine, the nucleoside of adenine arising from degradation of nucleotides or from S-adenosyl-homocysteine, can be treated in that manner.

The pentose phosphate cycle is under both coarse (slow) and fine (rapid) control. Some carbohydrates coming to the liver in excess of the amount that can be deposited as glycogen or utilized in glycolysis can cause adaptive changes in the liver resulting in an increased flux of glucose 6-phosphate through the pentose phosphate cycle. Those changes are mediated by as much as a tenfold increase in synthesis of two enzymes of the oxidative branch of the cycle, glucose 6-phosphate dehydrogenase and phosphogluconate dehydrogenase; the increase occurs maximally at about 3 days of high-carbohydrate feeding (155).

Fine control is exerted at the level of the reaction catalyzed by glucose 6-phosphate dehydrogenase; this reaction is irreversible in the presence of the lactonase and is rate-limiting for the entire sequence. NADPH, ATP, and long-chain acyl-CoA thioesters inhibit that enzyme (156,157). Complete inhibition of the dehydrogenase is considered to occur when NADPH is present in a concentration only one-third of that occurring in normal liver; accordingly, the enzyme would be in a state of complete inactivity under usual metabolic conditions. The question then becomes, How is that inhibition relieved? Withdrawal of NADPH to lower its concentration could achieve deinhibition; this could occur with NADPH utilization in synthesis of fatty acids (155). However, a second mechanism has been proposed (155) for relief of inhibition; this involves a reaction of the dehydrogenase with oxidized glutathione (GSSG) in cooperation with another, as yet unidentified, dialyzable factor. The regulation of the pentose phosphate pathway as a function of the NADPH/NADP⁺ ratio is supported by other experiments (158).

Glucose 6-phosphate, the substrate of the dehydrogenase reaction, is at the crossroads of three other pathways of carbohydrate metabolism in the liver: glycogen synthesis, glycolysis, and gluconeogenesis. The physiological significance of the rapid control mechanisms applied to the activity of glucose 6-phosphate dehydrogenase perhaps may be that inhibition shuts off access of glucose 6-phosphate to one of the pathways (the pentose phosphate cycle) and deinhibition opens it up (155). It is one way of specific channeling for the substrate; others are considered in the sections of this chapter on glycolysis and gluconeogenesis.

In the discussion of gluconeogenesis, a point was made of the remarkable fact that no accumulation of glucose 6-phosphate occurs in the livers of persons with type I glycogenosis owing to glucose 6-phosphatase deficiency. A notable feature of that disease, however, is hyperuricemia, which frequently results in gout (147,159---161). Although a contributing factor to the elevated blood level of uric acid is diminished secretion in the kidney, possibly because of competition by lactate, a major cause is considered to be increased *de novo* synthesis of purine nucleotides in the liver. Biosynthesis of purines is initiated with phosphoribosylpyrophosphate (PRPP), which serves as a scaffold for stepwise assembly of inosinic acid. In glucose 6-phosphatase deficiency, apparently glucose 6-phosphate is pushed through the pentose phosphate pathway concurrently with inhibition of gluconeogenesis, thus stimulating increased *de novo* synthesis of purine nucleotides. Because the latter synthesis occurs with obligate and coordinate utilization of ribose 5-phosphate to form PRPP, its regulation depends in the first instance on the activity of the pentose phosphate cycle.

GLYCOGEN METABOLISM IN THE LIVER

In the metabolism of glycogen in the liver, the immediate precursor that donates a glucosyl group to a growing chain of glycogen is uridine diphosphate glucose (UDP-glucose).

The priming mechanism for glycogen synthesis has been studied over many years, but only in the past decade has real understanding been achieved. Progress in that field has recently been reviewed (162). Although differences exist between the glycogens of skeletal muscle and liver, especially with regard to the nature of glycogen particles, the *de novo* synthesis of glycogen molecules in the two tissues requires similar priming mechanisms and almost identical priming proteins. The priming protein, called glycogenin, contains a single tyrosine residue that receives a glucosyl residue from UDP-glucose under the direction of a protein tyrosine glucosyltransferase. The glucan chain is then extended by about seven more glucosyl units from UDP-glucose by the autocatalytic action of glycogenin acting as a transferase. The glucosylated primer is then elongated by glycogen synthase. For this to happen, glycogenin and the synthase must be present as a complex. Liver glycogenin occurs to the extent of about 1/200th the amount of glycogenin found in skeletal muscle, so its presence is hard to detect. The significance of this difference in amount remains to be understood.

UDP-glucose, the glucosyl donor for glycogen synthesis, is formed from glucose 1-phosphate, which in turn arises from glucose 6-phosphate in a reaction catalyzed by phosphoglucomutase. Glucose 6-phosphate, in hepatocytes, can form directly from glucose in a reaction with ATP catalyzed by glucokinase or hexokinase.

Other hexoses, including fructose and galactose, ultimately can be isomerized to glucose in the form of glucose 6-phosphate, so that they too are capable of contributing to the synthesis of glycogen. Finally, all the gluconeogenic substances, including most amino acids, can contribute carbon atoms to the synthesis of glucose 6-phosphate and, therefore, are potentially glycogenic.

Formation of glycogen is in general favored by insulin and glucocorticoids (163,164). Formerly the hepatic glycogenic function of glucocorticoids was thought to be mediated through secretion and action of insulin. However, glucocorticoids now are known to induce glycogen synthesis in isolated hepatocytes, ruling out insulin as mediator (165). Indeed, injection of prednisone in animals causes a decrease of plasma insulin levels. Glucocorticoids cause changes in the activities of protein phosphatases that could result in increased glycogen deposition. Thus the steroids cause a significant increase in phosphorylase-phosphatase activity, thereby decreasing glycogenolysis (165). Furthermore, after treatment with glucocorticoids, glycogen synthase-phosphatase activity is less inhibited by phosphorylase; that would increase glycogen synthesis, since the unphosphorylated (i.e., dephosphorylated) form of the synthase is active.

In addition to regulation by hormones, deposition of glycogen is controlled by glucose per se. Glucose inhibits phosphorylase and stimulates glycogen synthase (165,166).

After a meal containing carbohydrate, the liver can deposit as much as 7% of its weight as glycogen. That, of course, does not represent the full potential of hepatocytes for storage of glycogen, since the feeding of protein-free, glucose-rich diets can cause that figure to reach about 10% (167); and, in glycogen storage diseases as, for example, type I glycogenosis, the figure can reach about 17% (168). In the normal case, however, soon after cessation of feeding, in the so-called postabsorptive period, glycogenolysis increases so that a major part of the glycogen store is depleted in about 4 hours; after 24 hours, less than 1% of liver weight is glycogen (130).

The cost in energy for the storage of glucosyl units into glycogen is modest relative to the functions served. As indicated, having a supply of glycogen in the liver allows the organism, in the postabsorptive state, to provide glucose for other tissues that are either obligate or facultative users of that fuel. Casting of glucose into its polymeric form allows a relatively large amount to be stored in hepatocytes without loss by diffusion and without the osmotic consequences that would attend the presence of high concentrations of the monosaccharide. Beginning with glucose and proceeding to formation of UDP-glucose, the activated intermediate that can transfer a glucosyl unit to a growing chain of glycogen with little loss of free energy, the equivalent of only one high-energy bond of ATP is used for glycogen synthesis; two such bonds should be counted if one considers the enzymatic hydrolysis of inorganic pyrophosphate formed in the reaction between UTP and glucose 1-phosphate. In the repayment of that energy, glycogenolysis utilizes inorganic phosphate to make glucose 1-phosphate, which is next converted to glucose 6-phosphate, thus making the pivotal phosphorylated intermediate of carbohydrate metabolism without the direct investment of energy from ATP. If glucose 6-phosphate is then hydrolyzed by glucose 6-phosphatase, and the glucose exits the hepatocyte into the peripheral circulation, no additional input of energy will have been required beyond the initial investment considered above. For cells such as the erythrocytes that receive the glucose, the liver will have supplied the energy that allows them to get a maximum net production of ATP from glycolysis.

The erythrocyte, in fact, is a special case in its dependence on the liver. Red blood cells store little if any glycogen, use almost solely glucose for energy, and obtain that energy only by glycolysis, since they have neither a tricarboxylic acid cycle nor an electron transport system. Thus the two net molecules of ATP coming from glycolysis represent the major energy supply of the erythrocyte. If it stored glycogen, the erythrocyte could lose the equivalent of one of those molecules of ATP as noted previously. Instead, the liver has paid the energy of storage. Furthermore, the lactate formed in glycolysis in the red blood cells is carried to the liver where, by gluconeogenesis involving the utilization of four molecules of ATP and two of GTP, it is converted to glucose, which once again goes to the erythrocyte. That example demonstrates how, in matters of energy, other tissues may depend on the liver.

On the other hand, when glucose 6-phosphate arising from glycogenolysis is used in the liver itself, not only is the energy of glycolysis obtained as ATP, but considerably more ATP may result if the acetyl-CoA formed by oxidation of pyruvate is sent through the tricarboxylic acid cycle and the respiratory chain. Under some metabolic circumstances, however, the acetyl-CoA may be used preferentially for synthesis of fatty acids.

The hormonal control of glycogen metabolism, mediated through cyclic nucleotide systems or calcium ion fluxes or both, has been reviewed extensively with particular emphasis on phosphorylation---dephosphorylation modifications of the enzymes concerned (165,166,169---188). Although much of the experimental work reviewed deals with metabolism of glycogen in muscle, several of these excellent articles are concerned specifically with events in the liver (165,166,172,174,180,184---189). The following discussion highlights some of those events.

Glycogen synthase is the enzyme governing the transfer of glucosyl units of UDP-glucose to a growing chain of glycogen, forming new α -1,4-glucosidic linkages. The enzyme occurs in a nonphosphorylated active form, designated *a*, which can be converted to a phosphorylated inactive form, designated *b*. The conversion is catalyzed by any one of a number of protein kinases utilizing ATP. A phosphatase may hydrolytically cleave the phosphate group of *b*, thus restoring the enzyme to the *a* species.

In contrast, phosphorylase, the enzyme that catalyzes glycogenolysis, occurs in an active phosphorylated form, called *a*, which can be cleaved hydrolytically by a phosphatase to an inactive, nonphosphorylated form, called *b*. The enzyme catalyzing the phosphorylation of glycogen phosphorylase is phosphorylase kinase, which exists as an inactive unphosphorylated *b* form and is converted into fully active *a* form by a cyclic AMP---dependent protein kinase; phosphorylase kinase also phosphorylates glycogen synthase *a* to render it inactive. Thus, any hormone or effector, such as glucagon, that causes the production of cyclic AMP and thereby the activation of the cyclic AMP---dependent protein kinase, will stimulate glycogenolysis and reciprocally inhibit glycogen synthesis.

Recently work from several laboratories (reviewed in refs. 185,187,190) has placed great emphasis on the major role of protein phosphatases in controlling the extent and specificity of protein phosphorylation, and thus in regulating particular physiological processes such as the synthesis of glycogen and glycogenolysis. That work has led to the proposal of a new mechanism for regulation of protein phosphorylation in which the protein phosphatase contains a targeting subunit (T_{sub}) in addition to its catalytic subunit (C_{sub}). T_{sub} localizes (i.e., bridges) the C_{sub} of the phosphatase to its target locus; this allosterically alters the properties of C_{sub} to increase its activity (dephosphorylation) (190). In the specific case of liver glycogen, in analogy to what happens in muscle, the following would occur. Glycogen binds three enzymes involved in its metabolism: glycogen synthase, glycogen phosphorylase, and phosphorylase kinase. All of these enzymes are controlled by reversible phosphorylation. In order to act on those bound enzymes, $PP1_G$ (the protein phosphatase involved in regulation of glycogen metabolism) binds to its target glycogen by means of its targeting subunit (a specific T_{sub} called G_{sub}), thereby allosterically altering its C_{sub} , augmenting its phosphatase activity, and causing its bound substrates (particularly the phosphorylase kinase and glycogen synthase) to be dephosphorylated. Other potential phosphorylated protein substrates would not be dephosphorylated since they do not bind to glycogen; thus specificity is achieved for colocalized substrates. Hormones can modulate this activity by controlling the phosphorylation by specific protein kinases of different sites on $PP1_G$.

In the case of insulin acting in muscle, for instance, an insulin-stimulated protein kinase promotes phosphorylation of a specific site on the G_{sub} of $PP1_G$, thereby enhancing the phosphatase activity toward the bound glycogen synthase and phosphorylase kinase. Thus the respective rates of activation and inactivation of these enzyme substrates are considered to define the reason why insulin stimulates the synthesis of glycogen.

The control of glycogen synthesis and glycogenolysis has been most clearly delineated for skeletal muscle. Nevertheless, the processes of glycogen metabolism appear to be similar in the liver, with some notable differences occurring with respect to hormonal control (165,166,185,190).

A variety of hormones and peptides, in concentrations of 10^{-7} to 10^{-11} M, can promote glycogenolysis. They act by binding to specific receptors on the surface of the hepatocyte, an event that triggers one or another transducing system, that is, a system that couples the binding of the hormone to production of a physiological result. In the liver, the transducing systems include (i) the cyclic AMP cascade that activates cyclic AMP---dependent protein kinases (191); (ii) the calcium---calmodulin system that activates other protein kinases (192); and (iii) the various phosphatidylinositol pathways producing phosphoinositols and diacylglycerols that activate a cyclic AMP---independent protein kinase called protein kinase C (193---196). Some hormones recognize two different types of receptors. For example, vasopressin in liver cells binds to a receptor that couples with a calcium second messenger system acting through the phosphatidylinositol-transducing system to produce its physiological functions, including glycogenolysis; the same hormone, however, in some kidney cells, binds to a receptor that couples with the cyclic AMP system to produce its water and mineral effects (196,197).

In the last edition of this treatise we called attention to a then new finding that glucagon may operate in liver cells through two separate receptors, one coupling to a transducing system resulting in an increase of cyclic AMP and a second coupling to a system resulting in an increase of intracellular calcium and activation of protein kinase C (198,199). Both signals would result in the known effects of glucagon to stimulate glycogenolysis and gluconeogenesis. A very recent paper (200) reports the expression cloning of the rat liver glucagon receptor and describes its signaling properties. A clone of the complementary DNA for the glucagon receptor was isolated and expressed in several kidney cell lines. The single receptor was able to transduce signals that caused an accumulation of two different second messengers, cyclic AMP and calcium. Attempts to find a second receptor for glucagon were unsuccessful. Thus the glucagon receptor resembles the receptors for calcitonin and parathyroid hormone in that binding of the hormone can lead to accumulation of two different second messengers, cyclic AMP and calcium. Of further interest is that the predicted amino acid sequence of the glucagon receptor, arising from the DNA sequence, shows sequences identical with some found in the receptors for calcitonin, parathyroid hormone, and secretin. The authors suggest alternative signaling pathways used by the

glucagon receptor may derive from its interaction with different G proteins as had been previously suggested (201).

Although glucagon may promote glycogenolysis by the mechanisms just discussed, the actual output of glucose from the liver may be partially suppressed by the presence of insulin. Thus the ratio of glucagon to insulin may determine whether glycogenolysis results in hyperglycemia.

Epinephrine, which promotes glycogenolysis in muscle by binding to β -receptors and thereby triggering the cyclic AMP cascade, may not act in that way in hepatocytes of all species. It appears to act through β -receptors in the liver of the dog, but in the liver of the rat and the human, catecholamines probably act through binding to α -receptors. In the latter case, the effect on glycogenolysis probably is mediated primarily through changes in concentration of calcium ions and not through the cyclic AMP system (140,180).

Other polypeptides that stimulate glycogenolysis in the liver are vasopressin, angiotensin II, and oxytocin. Their effects do not occur by initiation of a cyclic nucleotide system; they may act by causing movement of calcium ions.

Regardless of the mechanism used to transduce the glycogenolytic effect of a hormone or peptide, the final result is an activation of phosphorylase. Under most conditions, sufficient active form of phosphorylase is present in the liver to catalyze a significant degree of glycogenolysis, but its action is exalted or damped by various noncovalent modifiers. AMP (adenylate) activates phosphorylase *b* but also inhibits the phosphatase that inactivates phosphorylase *a*. Its overall effect, then, is to sustain glycogenolysis. Under anaerobic or anoxic conditions, AMP accumulates in the liver at the expense of ATP and ADP, and glycogenolysis accordingly is promoted. In contrast, the presence of a large concentration of glucose can inhibit both forms of phosphorylase and stimulate phosphorylase *a* phosphatase; the net result is inhibition of glycogenolysis, as occurs in the liver shortly after ingestion of a meal containing relatively large amounts of glucose.

In addition to the release of glucose from glycogen through the agency of phosphorylase, phosphoglucomutase, and glucose 6-phosphatase, additional formation may occur through the action of hydrolytic enzymes classified as amylases. Two principal amylases are the amylo-1,6-glucosidase and the lysosomal acid α -1,4-glucosidase.

In the last 10 years or so, a number of related articles (120,188,202---212) have dealt with the synthesis of glycogen in the livers of rats under conditions of fasting followed by refeeding with glucose or sucrose. The results offer some unusual new insights into the pathways by which the carbon atoms of glucose ultimately become incorporated into glycogen. In those experiments, when animals were fasted and then refed with glucose suitably labeled with ^{14}C or ^3H or both, the patterns of isotope found in the glucosyl units of glycogen isolated from the livers appeared to indicate that (i) about one-third of the glycogen had formed by the so-called direct pathway from a pool of glucose 6-phosphate made by phosphorylation of glucose; (ii) the remaining approximately two-thirds had formed "indirectly" from a second pool of glucose 6-phosphate made by gluconeogenesis from 3-carbon compounds such as pyruvate and lactate. (More recent experiments indicate that the extent of synthesis of glycogen by the indirect pathway may vary depending on conditions, and may perhaps be about one-half instead of two-thirds.) Although this result appears surprising, it would be consistent with the observations by some that the liver has a limited capacity for phosphorylating glucose with hexokinase or glucokinase (105,210), so that much of dietary glucose can exit the liver without ever having become phosphorylated, be carried to extrahepatic tissues such as skeletal muscle and erythrocytes, and then by glycolysis become converted to lactate. The lactate would then return to the liver to be converted by gluconeogenesis to glucose 6-phosphate and glycogen. However, other investigators (186,211) have provided evidence that phosphorylation of glucose may not be limiting. Some (209) have stated that glycogen forming in the liver by gluconeogenesis may come mainly from 3-carbon compounds made in the liver rather than in the periphery. Those authors prefer the concept that the liver simultaneously engages in glycolysis and gluconeogenesis and that hepatic zonation and bidirectional substrate cycling may explain the nature of glycogen formed after feeding of a carbohydrate meal. The relationship of glycolysis, gluconeogenesis, and glycogen synthesis with possible metabolic zonation in the liver is discussed under "Metabolic Zonation" below.

CONVERSION OF CARBOHYDRATE TO FAT

Based on common human dietary experience as well as on experiments in animals, lipogenesis from carbohydrate has long been considered a significant metabolic pathway in humans. Just as in recent years our views on glycogen synthesis directly from glucose have had to be revised to include a significant indirect pathway, new considerations and methods of study (213---215) are currently raising questions about the extent of lipogenesis from carbohydrate in humans. Using methods employing stable isotopes, investigators have found insignificant *de novo* synthesis of fatty acids from a heavy carbohydrate load in normal, nonoverfed, nonobese men (214,215).

In those experiments, dietary fat and not glucose was the precursor material for fatty acids found in the circulation and in adipocytes. The authors explain that the observed hypertriglyceridemia could be due to inhibition by excess carbohydrate of hepatic fatty acid oxidation and to increased hepatic re-esterification of fatty acids. Nevertheless, relative to this question, many animal (especially rat) studies described in this chapter demonstrate (or assume) significant conversion of carbohydrate to fat.

FATTY ACID METABOLISM AND KETOGENESIS

The pathways in the liver for biosynthesis of fatty acids in the cytosol and of fatty acid oxidation and ketogenesis in the mitochondria are discussed in several excellent reviews (216---231). The biosynthesis of unsaturated fatty acids in liver, and its control, also have been reviewed (226,228---230,232).

Normally, the liver obtains most of its energy by metabolism of fatty acids. When an animal ingests carbohydrate in large excess, the liver responds with increased lipogenesis; both fatty acid and glycerol moieties can be derived from absorbed hexoses. The newly formed triacylglycerols are incorporated into lipoproteins and sent into the peripheral blood; if the rate of lipogenesis exceeds that of lipoprotein synthesis, excess triacylglycerols are stored within liver cells in membrane-enclosed cytoplasmic vesicles (233). During a subsequent restriction of carbohydrate, the triacylglycerols in the vesicles undergo lipolysis, perhaps with the participation of lysosomal enzymes (233). Accordingly, fatty acids are released to be taken up by the mitochondria (234), where they are oxidized with formation of acetyl-CoA and ketone bodies. One should note that hepatocytes can form triacylglycerols utilizing either exogenous fatty acids or those synthesized *de novo* in the liver.

In periods of short supply of carbohydrate in tissues, as in fasting, starvation, and diabetes mellitus, lipogenesis is repressed, glycolysis is inhibited, and oxidation of fatty acids is greatly stimulated. A prodigious formation of acetoacetate and its reduced derivative, D-3-hydroxybutyrate, ensues. In the first instance, the ketogenesis is made possible by the availability of fatty acid substrates; these arise in adipose tissue by lipolysis with a hormone-sensitive lipase (235) and are brought to the liver in the form of complexes with albumin.² In the physiological and pathological states of carbohydrate deprivation mentioned above, however, changes occur in the concentrations of glucagon and insulin in the blood and, accordingly, in their ratio. That appears to be a major mechanism by which the liver becomes a ketogenic organ, since glucagon in an imbalance with insulin represses glycolysis and causes decreased production of malonyl-CoA, oxaloacetate, and citrate. How diminished concentrations of those metabolites channel acetyl-CoA into the ketogenic pathway is considered below. Since most of those effects can be reproduced experimentally with dibutyryl cyclic AMP (220,236,237), glucagon has been considered to act by increasing the synthesis of cyclic AMP in hepatocytes; however, this matter should be thought over because of new evidence that the glucagon receptor can also couple to a protein kinase C system (199; see also 238 and 200).

The remarkable capacity of the liver to oxidize fatty acids and produce acetoacetate is emphasized in an estimate made (218) that the liver, in 1 day, can synthesize half its weight of ketone bodies (approximately 900 g/day). That seemingly exorbitant potential probably is tapped only in a few conditions, such as severe diabetes. One may compare that amount with the quantity of glucose that the liver can produce in a day of fasting after a meal that had resulted in storage of glycogen at a level of about 7%; first about 150 g glucose would result from glycogenolysis, followed by perhaps an equal quantity from gluconeogenesis from lactate.

Acetoacetate is neither a product of incomplete oxidation of fatty acids nor a waste product of fat metabolism. It is formed from 3-hydroxy-3-methylglutaryl-CoA, which in turn is formed from the reaction of acetoacetyl-CoA and acetyl-CoA; as seen in Fig. 8, acetyl-CoA can form at all stages of β -oxidation of fatty acids. Acetoacetate is generated in response to signals from extrahepatic tissues mediated by glucagon and insulin informing the liver that glucose must be conserved for its obligate users and that other tissues are prepared to use the ketone bodies for energy. Subsequently, the ketone bodies, acting in a feedback mechanism, may modulate the release of fatty acids from adipose tissue and the turnover of protein in muscle (219,240,241).

As already stated, the regulation of fatty acid oxidation and ketogenesis in liver has been studied extensively. Some attempts have been made to understand that regulation in terms of the redox state of hepatocytes as reflected in the ratio of NAD^+/NADH ; the increased oxidation of fatty acids would result in a decrease of this ratio, theoretically causing a shift of the reaction catalyzed by malate dehydrogenase in favor of malate production at the expense of oxaloacetate (242,243). Another way in which the concentration of oxaloacetate in hepatocytes could be decreased is by diversion of that metabolite into the pathway of gluconeogenesis (244). In both cases, diminished concentration of oxaloacetate was postulated to result in diminished synthesis of citrate, the precursor of fatty acid synthesis. Some investigators, however, have obtained evidence that neither of those explanations can rationalize all the ketogenic situations encountered experimentally and clinically (218,220).

Accordingly, two additional but not mutually exclusive hypotheses have been proposed for control of fatty acid oxidation and ketogenesis when carbohydrate is in short supply. One proposal (220) accepts the concept that oxaloacetate becomes the limiting substrate but pinpoints the cause more remotely in the glycolytic pathway at the level of the 6-phosphofructokinase reaction. Deactivation of that enzyme would diminish the flux of substrate through glycolysis, decrease the amount of pyruvate formed, and hence reduce the amount of oxaloacetate that can form from pyruvate. The second proposal (218) places the control of fatty acid oxidation and ketogenesis at the level of transport of fatty acids into the mitochondria, more specifically in the activity of carnitine acyltransferase I. Both proposed mechanisms are considered to be regulated strongly by glucagon.

With respect to the first proposal, focus is placed on the branch point of reactions of acetyl-CoA in the mitochondria (220). Acetyl-CoA in the mitochondria has the option of condensing with oxaloacetate to form citrate; the citrate then can exit to the cytosol where, at the site of fatty acid synthesis, it can be cleaved to yield acetyl-CoA once again. Alternatively, acetyl-CoA in the mitochondria can form 3-hydroxy-3-methylglutaryl-CoA, which can be cleaved to form acetoacetate. Thus at the fork indicated above, the pathway taken by acetyl-CoA could be determined by the concentration of oxaloacetate.

It was found that either dibutyl cyclic AMP or glucagon caused chick liver cells to suffer precipitous diminution of cytosolic citrate and of mitochondrial oxaloacetate; this was coincident with decreased synthesis of fatty acids and increased ketogenesis (220). Decrease of citrate concentration in the cytosol might be expected to reduce the activity of the enzyme that catalyzes the committed and rate-limiting step in fatty acid synthesis, namely, acetyl-CoA carboxylase; the reaction involved is the carboxylation of acetyl-CoA to form malonyl-CoA. It has been thought that the carboxylase is active only when it is in a polymeric, filamentous form, as favored by citrate, and that it is inactive when depolymerized, as in the absence of citrate. Thus in that view citrate is both the precursor for acetyl-CoA and the allosteric activator for conversion of acetyl-CoA to fatty acids. However, more recent evidence indicates that citrate has no effect on the fully active unphosphorylated form of the carboxylase (245).

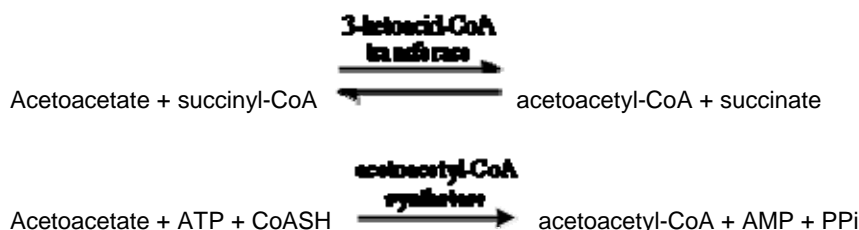
In the experiments described above, the fall in citrate level could be overcome by addition of pyruvate or lactate; concomitantly, the inhibitory effects on fatty acid synthesis and the activating effects on ketogenesis were reversed. The effects of cyclic AMP or glucagon noted previously must then have been mediated in glycolysis at some point before formation of pyruvate. The actual locus was determined to be at the level of the reaction catalyzed by 6-phosphofructokinase. Whether fructose 2,6-bisphosphate (the principal activator of 6-phosphofructokinase, whose synthesis is inhibited by glucagon) can stimulate fatty acid synthesis and inhibit ketogenesis remains to be determined.

The proposal of Lane and Mooney (220) can be summarized as follows: Carbohydrate feeding promotes glycolysis with formation of pyruvate, the major anaplerotic (replenishing) precursor of oxaloacetate in the liver. Oxaloacetate produced in the mitochondria reacts with acetyl-CoA to form citrate; citrate is sent into the cytosol, where, acting as both substrate and allosteric regulator, it favors fatty acid synthesis. Diversion of acetyl-CoA into that pathway, which depends on an adequate supply of oxaloacetate, tunes down the entry of acetyl-CoA into the ketogenic pathway. Glucagon, operating through cyclic AMP, reverses those processes by limiting the formation of pyruvate in glycolysis.

McGarry and Foster (218,237), in whose laboratories the alternative proposal of regulation was developed, place the control by carbohydrate of fatty acid synthesis and ketogenesis at the point of entry of fatty acids into the mitochondria where they can be oxidized (234). Under conditions of carbohydrate feeding, increased glycolysis occurs, more acetyl-CoA forms from pyruvate, and more malonyl-CoA forms from the acetyl-CoA. Malonyl-CoA has a direct inhibitory effect on the carnitine acyltransferase I reaction, thus preventing the transport of long-chain fatty acids into the mitochondria and, therefore, their oxidation to acetyl-CoA and subsequent formation of ketone bodies. Glucagon, or dibutyl cyclic AMP, activates the translocation of long-chain fatty acids across the inner mitochondrial membrane. This is thought to occur by an inhibition of acetyl-CoA carboxylase, the enzyme that catalyzes formation of malonyl-CoA. The sequence of events is then as follows: Carbohydrate feeding causes increased production of pyruvate and, therefore, of malonyl-CoA. Malonyl-CoA inhibits the translocation of long-chain fatty acids into the mitochondria by inactivating the carnitine acyltransferase. Thus fatty acid oxidation and ketogenesis in the mitochondria are inhibited, while fatty acid synthesis can proceed in the cytosol. Should a glucagon---insulin imbalance occur, as when carbohydrate feeding or utilization is curtailed, glucagon causes deactivation of acetyl-CoA carboxylase (245---251), production of malonyl-CoA is diminished, inhibition of translocation of long-chain fatty acids is relieved, and fatty acids enter the mitochondria and are oxidized with formation of acetyl-CoA and, subsequently, ketone bodies.

Although the liver is the major organ for production of ketone bodies, it has little mitochondrial 3-ketoacid-CoA transferase, one of the two enzymes that allows a tissue to utilize acetoacetate for energy; thus the liver does not itself oxidize nonthiolated acetoacetate to a significant degree. However, an acetoacetyl-CoA synthetase has been located in the cytosol of liver cells; this is devoted primarily to synthesis of cholesterol and fatty acids (252). In many peripheral tissues, including brain, heart and skeletal muscle, kidney, and intestine, acetoacetate may be

prepared for oxidation by the following set of reactions, although the activity of 3-ketoacid-CoA transferase is generally more than tenfold greater than that of acetoacetyl-CoA synthetase:



Acetoacetyl-CoA is acted on by acetoacetyl-CoA thiolase to give acetyl-CoA for entrance into the tricarboxylic acid cycle. That enzyme is present in liver but, because of the absence of 3-ketoacid-CoA transferase, is used mainly in the reverse reaction, as required in the process of ketogenesis:

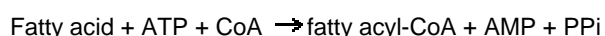


The ketone bodies have been considered to be more than substrates for oxidation in certain peripheral tissues. Emphasis has been placed on acetoacetate and 3-hydroxybutyrate as signals of carbohydrate lack and, therefore, as agents for integration of different fuels for use in the entire body (219,241).

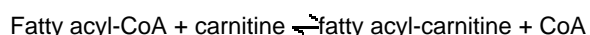
PEROXISOMAL OXIDATION OF FATTY ACIDS

In 1969, studies on the endosperm of germinating castor bean (255,256) described a nonmitochondrial system of β -oxidation of fatty acids occurring in the class of microbodies known as glyoxysomes. The authors proposed a pathway involving a fatty acyl-CoA oxidase different from the fatty acyl-CoA dehydrogenase present in mitochondria of many tissues, although both enzymes are FAD linked. The investigators noted a major difference in the way the reduced FAD is reoxidized. In mitochondria, the enzyme couples with the electron transport chain, eventually passing the hydrogen atoms and electrons of FADH_2 to oxygen to form water; in the peroxisomes, the oxidase couples directly with molecular oxygen, passing hydrogen atoms and electrons through to form hydrogen peroxide; hydrogen peroxide would then be removed by the action of catalase.

Subsequently, similar systems of fatty acid oxidation were described in protozoan ciliates (257---259). It was then established (260,261) that such systems were operative in liver peroxisomes, and the pathway was formulated in considerable detail. Others (262---267) studied the system in livers of several species, including the human (268). Especially important was the finding that administration of clofibrate or other hypolipidemic drugs to animals caused their livers to respond with induction of enzymes of the peroxisomal system of fatty acid oxidation (261,266---273). Both pathways are initiated with an investment of energy from ATP to activate the carboxyl group of a fatty acid, in the following reaction:



The reaction is catalyzed by an enzyme called fatty acyl-CoA synthetase. One species of the enzyme is linked with the mitochondrial system of fatty acid oxidation, and the locus of its action in the cytosol makes necessary a means of translocation of the activated fatty acid into the mitochondria. This is accomplished by the formation of fatty acyl-carnitine derivatives catalyzed by a carnitine fatty acyl-CoA transferase located in the inner membrane of the mitochondria:



In the matrix of the mitochondria, then, the fatty acyl group is again transferred to CoA to make fatty acyl-CoA that can be oxidized. In contrast, the translocation of the fatty acid into peroxisomes is independent of carnitine, because the fatty acyl-CoA synthetase to which it is linked is located in the peroxisomes per se. Thus the activated fatty acyl-CoA is generated within the organelle in which its oxidation is to occur. Apparently, however, the translocation of the free fatty acid from the cytosol into the peroxisome requires the participation of a fatty acid binding protein (274).

From the standpoint of energy metabolism, the differences in the first oxidative step between mitochondrial and peroxisomal pathways are critical. The mitochondrial enzyme is an FAD-linked fatty acyl-CoA dehydrogenase

that couples with the electron transport chain, so that the energy released in the subsequent reoxidation of the reduced FAD is preserved in pyrophosphate bonds of ATP; in the course of that reoxidation, hydrogen atoms and electrons of FADH₂ are transferred to oxygen to form water. In peroxisomes, the enzyme catalyzing the oxidation of fatty acyl-CoA is an FAD-linked oxidase; reoxidation of the reduced flavin occurs with molecular oxygen without participation of a respiratory chain. Thus no ATP is produced, and the hydrogen atoms of FADH₂ are passed on to oxygen to produce hydrogen peroxide. The latter substance may be decomposed by the action of catalase also present in the peroxisomes, although, alternatively, the catalase---hydrogen peroxide complex could oxidize a second substrate, such as ethanol or methanol, if indeed such were present. Because of the two different ways by which the FADH₂ coenzyme is reoxidized, the mitochondrial dehydrogenase is cyanide sensitive, while the peroxisomal oxidase is cyanide insensitive.

Proceeding down the pathway, the next enzymes show significant differences as between the two organelles. In the mitochondrial pathway, the enoylhydratase and L-3-hydroxy fatty acyl-CoA dehydrogenase activities are exhibited by separate enzymes. In the peroxisomal pathway, the two activities are associated with a single bifunctional enzyme. In the mitochondria, NADH produced by the dehydrogenase can enter the respiratory chain and yield three molecules of ATP by oxidative phosphorylation. NADH produced in the dehydrogenation reaction in peroxisomes must be shuttled out. Perhaps eventually its reducing equivalents would be transferred to the mitochondria; depending on the shuttling mechanism employed, either two or three molecules of ATP could be produced.

Another difference between the two systems is exhibited by the corresponding enzymes catalyzing the thiolitic cleavage of 3-keto fatty acyl-CoA to yield acetyl-CoA. The mitochondrial and peroxisomal thiolases differ in specificity and other properties.

Hepatic peroxisomal β -oxidation is specific for long-chain fatty acyl-CoA derivatives, whereas the mitochondrial system utilizes both long- and short-chain fatty acyl-CoA substrates. However, the peroxisomal system appears to be most active with fatty acids containing 10 to 22 carbon atoms and is particularly active with monounsaturated fatty acids, such as erucic acid (C22:1) (31,254,275).

Peroxisomal oxidation of long-chain fatty acids stops at the level of octanoyl-CoA, whereas mitochondrial oxidation continues to formation of acetoacetyl-CoA. The further oxidation of octanoyl-CoA could then proceed by one of two mechanisms. First, the substrate could be hydrolyzed by a hydrolase to form octanoic acid and CoA; the octanoic acid could diffuse out to the cytosol and again be activated for oxidation in the mitochondria. Although some studies have indicated that indeed peroxisomal hydrolases exist (276), another publication (277) reports that hydrolases separating with preparations of peroxisomes are probably contaminants arising from lysosomes. Accordingly, the hydrolytic mechanism is not viable. The second mechanism for the transfer of medium- and short-chain acyl groups out of peroxisomes could perhaps involve peroxisomal carnitine fatty acyl-CoA transferases. Indeed, these have been found to exist for the formation of acetyl-carnitine and octanoyl-carnitine. The carnitine derivatives could diffuse out to the cytosol and eventually transfer their fatty acyl groups to mitochondria for oxidation or be used in synthetic reactions. Although the oxidation of long-chain fatty acids in peroxisomes does not begin with a transfer reaction requiring carnitine, exit of the medium-chain fatty acyl groups formed in oxidation may require the participation of carnitine.

In the mitochondria, control is exerted on fatty acid oxidation at the level of the carnitine fatty acyl-CoA transferase reaction, which aids in translocation of the fatty acyl group; thus malonyl-CoA formed as a result of carbohydrate metabolism inhibits the transferase reaction (146,218,278) and, therefore, diminishes the oxidation of fatty acids. The peroxisomal system is free of such control and apparently is regulated only by the concentration of fatty acid substrate.

The energetics of oxidation are different in the two systems. In the peroxisomes, oxidation of palmitic acid (C¹⁶) would occur in four repetitive stages, with formation of octanoyl-CoA, four molecules of acetyl-CoA, and four of NADH. Those products would leave the peroxisomes by the mechanisms described previously and ultimately could be oxidized in the mitochondria to yield about 121 molecules of ATP. If palmitic acid were to be oxidized directly by the mitochondrial system, about 129 molecules of ATP would result. Carried to completion with subsequent help from the mitochondrial system, oxidation of palmitic acid in the peroxisomes produces only eight fewer ATP molecules. These eight are lost at the four steps in which the fatty acyl-CoA oxidase operates. That relatively small amount of energy would not be a great price to pay if use of the peroxisomal pathway confers some important advantages to the organism (31). Although the advantages are not known for the existence of the parallel peroxisomal pathway, partition of total oxidation between the two pathways has been studied: About 32% of the total oxidation of palmitate (in terms of acetyl-CoA formed) was found to occur in the peroxisomes of hepatocytes (279). A later estimate stated that peroxisomal fatty acid oxidation contributes no more than 10% to overall hepatic oxidation of palmitate plus oleate (267). It may be conjectured that the peroxisomal pathway provides a mechanism for the production of acetyl-CoA outside the mitochondria without the participation of citrate formed in the mitochondria. Furthermore, from knowledge obtained concerning induction of most of the

enzymes of the peroxisomal pathway by hypolipidemic drugs (261,269---273), and by excessive fat intake (280), even the marginal loss of energy occurring when that pathway is used instead of the mitochondrial system could, under certain circumstances, contribute to removal of lipid and loss of body weight. Finally, oxidation of fatty acyl-CoA derivatives by the oxidase allows generation of hydrogen peroxide, which may be used by catalase for oxidation of substrates such as ethanol.

OXIDATION OF PYRUVATE AND ACETYL-CoA

Lactate is produced in liver cells when glycolysis occurs anaerobically. That process results in net formation of two molecules of ATP for each molecule of glucose oxidized. The change in free energy (ΔG°) occurring when glucose is converted to two molecules of lactate is $-47 \text{ kcal}\cdot\text{mol}^{-1}$; that occurring when glucose is oxidized completely to carbon dioxide and water is $-686 \text{ kcal}\cdot\text{mol}^{-1}$. Thus glycolysis unlocks only about 6% of the total free energy available from glucose metabolized by the glycolytic, pyruvate dehydrogenase, and tricarboxylic acid systems. The total oxidation of glucose results in formation of about 36 to 38 molecules of ATP. One may then calculate the efficiency of energy preservation to be about 31% for anaerobic glycolysis and about 38% to 40% for the complete oxidation of glucose by the combined actions of glycolysis and the tricarboxylic acid cycle. Thus, although the oxidation of pyruvate yields many more times the amount of ATP than is produced in glycolysis, the efficiencies of the two pathways are not too disparate.

The oxidation of pyruvate is an aerobic process. Pyruvate formed in glycolysis enters the mitochondria, where it is oxidatively decarboxylated by the pyruvate dehydrogenase system. The acetyl-CoA that forms can then enter the Krebs tricarboxylic acid cycle, where two carbon atoms are oxidized to carbon dioxide. The energy of oxidation of pyruvate is preserved in the pyrophosphate bonds of ATP, as shown in Table 1. A single substrate-level phosphorylation in the tricarboxylic acid cycle results in formation of GTP.

Acetyl-CoA arising from fatty acid oxidation in the mitochondria can also enter the tricarboxylic acid cycle and be treated identically with acetyl-CoA coming from pyruvate. That is true also for acetyl-CoA derived from the oxidation of the carbon chain of leucine.

The tricarboxylic acid cycle is regulated by events in the pyruvate dehydrogenase system, which controls the conversion of pyruvate to acetyl-CoA. The subject of that regulation has been dealt with in several reviews (113,281---287). However, control of the cycle also depends on factors that determine how much acetyl-CoA will be produced from fatty acids and which of the metabolic options open to acetyl-CoA will be taken. Finally, control of the tricarboxylic acid cycle per se occurs at two major points. The first occurs at the level of citrate synthesis, at which citrate synthase responds exquisitely to the concentration of oxaloacetate and is inhibited by the concentration of citrate. The second control is with respect to isocitrate dehydrogenase, which is stimulated by ADP and inhibited by NADH.

The pyruvate dehydrogenase system consists of several different components that participate in three integrated reactions summed up in the following equation:



The reoxidation of NADH by the respiratory chain can give rise to three molecules of ATP. The continual functioning of the system, which requires reoxidation of NADH, thus depends on the presence of molecular oxygen. Failing that, pyruvate would be reconverted to lactate, and the full energy benefit of oxidation of carbohydrate would not be attained. The fine control of pyruvate oxidation, however, is achieved in several different ways, most of which relate to enzyme₁ of the complex. As the reaction proceeds, the ratios of acetyl-CoA/CoA and NADH/NAD⁺ increase coordinately, and the activity of enzyme₁ decreases by a kind of end product inhibition of the sequence of reactions. However, enzyme₁ is regulated mainly by alternate phosphorylation and dephosphorylation reactions catalyzed, respectively, by a protein kinase and a phosphatase. The kinase, which is independent of cyclic AMP, is not activated by glucagon. It is bound to the pyruvate dehydrogenase complex and uses ATP μ Mg as the phosphorylating substrate. Phosphorylation of enzyme₁ causes inactivation, and dephosphorylation results in reactivation. Pyruvate itself inhibits the kinase and therefore promotes the activation of enzyme₁; accordingly, it promotes flux of substrate through the pyruvate dehydrogenase system and the tricarboxylic acid cycle. In conditions such as starvation and diabetes, where oxidative carbohydrate metabolism would be diminished, some accumulation of lactate would occur, and fatty acid oxidation and ketogenesis would be favored.

METABOLIC ZONATION

Considerable evidence exists to show that all hepatocytes are not geared to perform identical functions. At any given time, metabolic events occurring in a single cell need not be identical, quantitatively or qualitatively, with events in any other hepatocyte. For instance, when one states on the basis of gross analysis that the liver of a fed animal contains 5% glycogen and 4% fat, the picture that comes to mind is that every hepatocyte reflects these percentages rather than that the percentages constitute an average of perhaps widely varying individual cell contents. However, much evidence shows that hepatocytes do not all have the same percentages of stored fuels, and that therefore rates of synthesis and utilization are not uniform among the cells. In that case the enzymes involved, and the controls imposed, must have a range of differences among hepatocytes. The locational and temporal differentiation of functions among the cells is called "metabolic zonation."

Functional differentiation among cells can be determined or influenced by such factors as spatial relations to blood supply, paths of diffusion of oxygen and other substrates, and gradients in concentrations of substrates and hormones created in the microcirculation by abstraction into cells in the path of blood flow. In addition to these dynamic determinants of functional zones is the anatomical location of cells determined during development (289). Also of consequence could be possible zonation with respect to kinds and numbers of hormone receptors; this is as yet hardly explored. Finally, the zonation itself could result in the activation of signal molecules within a cell; these then could communicate with neighboring cells through gap junctions known to exist between hepatocytes (298). In that way information regarding regulation of metabolism could be transmitted.

In the liver lobule, zonation can be considered in terms of periportal and perivenous regions. Of course the definition of zones is not precise, since the cells encounter a gradation of changes in environment rather than a real or discrete boundary. In regard to the microcirculatory unit of the lobule, at the start of the periportal zone the blood is mixed arterial-venous, and at the end of the perivenous zone the blood is venous. The partial pressure of oxygen in rat livers has been reported to grade from the periportal zone with a PO_2 of approximately 65 mm Hg to the perivenous zone with a PO_2 of approximately 30 to 35 mm Hg (299,300). In apparent concert with the higher oxygen tension in the periportal zone, oxidative energy metabolism also predominates in that zone (283,290,294). Moreover, enzymes of the tricarboxylic acid cycle and respiratory chain are mainly located in hepatocytes in the periportal zone; these enzymes include succinate and malate dehydrogenases and cytochrome oxidase. Then, again, enzymes of glycolysis are relatively more active in the perivenous zone where oxygen tension is lower than in the periportal zone (300,301).

That the gradient of oxygen partial pressures occurring with the microcirculation of the blood in the lobule may influence the metabolic events described is supported by results of experiments with isolated hepatocytes. Thus, a mixed population of hepatocytes in primary cultures, exposed to PO_2 levels similar to those characteristic of periportal blood in vivo, behave metabolically in some respects like periportal hepatocytes (300,301); exposed to PO_2 levels encountered in perivenous blood, they behave in some respects like perivenous hepatocytes.

In other experiments (302), livers of rats were perfused in antegrade and retrograde fashions, allowing the demonstration that metabolic zonation could be reversed in vivo. For instance, perivenous cells in a liver that was perfused retrogradely quickly switched from the glycolytic mode while periportal cells became more glycolytic. Levels of PO_2 can influence the phosphate potential of a cell, that is, the relative concentrations of ATP, ADP, AMP, and Pi. Indeed, that group of investigators considered that levels of ADP might be critical in determining whether cells are glycolytic (302). One would expect that high levels of PO_2 would favor the oxidation of substrates through the pyruvate dehydrogenase system and the tricarboxylic acid cycle with concomitant formation, in associated reactions, of GTP and ATP. In that case, gluconeogenesis, an energy-requiring process, would be favored over glycolysis.

The hepatocytes in the periportal zone are more devoted to gluconeogenesis than those in the perivenous zone (300,303) and, under favorable nutritional circumstances, exhibit more of those enzyme activities related to gluconeogenesis either directly (glucose-6-phosphatase and phosphoenolpyruvate carboxykinase) or indirectly [tyrosine aminotransferase (304---306)]. On the other hand, perivenous hepatocytes are more active in glycolysis than are periportal ones (300,301). Although glycogen formation occurs more actively in the periportal zone than in the perivenous zone, it does occur to differing extents in both regions (292,307). This depends partly on dietary conditions of the animal, whether diets have been relatively rich in carbohydrate or in protein. In all of the glycogen-forming cells, one may observe clumps of glycogen in association with elements of the smooth endoplasmic reticulum (292,308,309). The clumps are of a different character in periportal as compared with perivenous cells, but the significance of this observation is not entirely clear.

Since gluconeogenesis predominates in the periportal zone, and glycogen formation also occurs there in a major way, one may consider whether a relationship exists between the two processes. Indeed, that is the case. A large portion of glycogen in the liver forms from glucose 6-phosphate generated from 3-carbon compounds traversing the gluconeogenic pathway. Accordingly it is consistent that glycogen formation and gluconeogenesis should occur in cells in the same zone.

Cells in the periportal zone as compared to those in the perivenous zone also are more active in glycogenolysis soon after receiving appropriate hormonal signals, although eventually perivenous hepatocytes also degrade their glycogen. In the whole animal, of course, the amount of glycogen accumulated in the cells is related to previous diet, and the nature of hormonal signals for glycogenolysis depends on the time after the last meal. In postprandial periods, when blood glucose levels fall off and insulin levels decrease, the pancreatic α -cells will secrete glucagon that will enter the portal circulation. At some point of postprandial time, the glucagon-to-insulin ratio will be relatively high in the periportal region, and this will favor gluconeogenesis and glycogenolysis. As the blood traverses the lobule, the periportal cells presumably bind glucagon so that the concentration of the hormone offered to perivenous cells downstream will be relatively less, and gluconeogenesis and glycogenolysis will diminish. Again, in theory, under the conditions described this would seem to require that the glucagon-to-insulin ratio be higher in periportal as opposed to the perivenous region.

If, as discussed in the section above on "Glycogen Metabolism in the Liver," the conversion of exogenous glucose to the 3-carbon compounds used in gluconeogenesis occurs mainly in peripheral tissues, but the gluconeogenic conversion of the 3-carbon compounds to glucose 6-phosphate occurs in the liver, one should expect this to be reflected in the levels of the most potent allosteric effector of gluconeogenesis, fructose 2,6-bisphosphate. When levels of this effector are relatively high, glycolysis is favored; when relatively low, gluconeogenesis is favored. Thus, in those experiments in which glucose was fed to rats that had previously been fasted (120,188,202---212), one should have expected that the levels of fructose 2,6-bisphosphate in the liver would be relatively low, reflecting the observed predominantly gluconeogenic mode. In fact, when tested, the levels were found to be relatively high, presenting what seemed to be a real paradox (310,311). To rationalize that finding, one can invoke the existence of unknown metabolic regulators, but that is unnecessary if one accepts the concept of metabolic zonation (311). Thus, if gluconeogenesis occurs principally in periportal hepatocytes, those cells would have low levels of fructose 2,6-bisphosphate, while much higher levels could occur in perivenous cells geared to glycolysis and not to gluconeogenesis. Analysis of a total liver sample might yield an average high value. This is not yet proved. Experiments would have to be performed showing zonation of the enzymes and their regulators engaged in synthesis and degradation of fructose 2,6-bisphosphate. One would also have to examine whether zonation exists with respect to concentration of the allosteric effector *per se*.

An attempt has been made to show zonation by differential destruction of either the afferent (perivenous) or efferent (periportal) part of the microcirculatory unit (312). This was done in rat livers using a digitonin-collagenase perfusion technique. By examining the activities of several enzymes and the rate of gluconeogenesis in the two kinds of hepatocytes, the authors were able to conclude that gluconeogenesis is more prominent in the periportal zone.

Other findings concerning location and activation of enzymes involved directly or indirectly in gluconeogenesis from amino acids are mostly consistent with the concept of metabolic zonation (313). Glutaminase, for example, an enzyme necessary in the series of reactions leading to conversion of carbon atoms of glutamine to those of glucose 6-phosphate, is principally located in the periportal zone (314). Of interest is that ureogenesis also is dominant in this region as opposed to the perivenous zone (314). On the other hand, glutamine synthase is located primarily in the perivenous zone. Interestingly, no hepatic zonation was found in a study of glutamine-transaminating activities (315).

The hepatic zonation of pathways of lipid metabolism have been recently reviewed (297). These pathways appear to be less zonated than those of carbohydrate metabolism, but some of the discrepancies found may be related to the methods used in their study.

Relative to the graded metabolic zonation in the liver, one is encouraged to draw an analogy with zonation as known to exist in a more discrete anatomical way in the kidney. Cells in the kidney cortex are poorly glycolytic and primarily gluconeogenic. Cells in the renal medulla are almost obligatorily glycolytic and are not gluconeogenic. Blood coming into the cortex is rich in oxygen and in substrates for gluconeogenesis; importantly among the latter is glutamine coming from the intestine, muscle, and liver. In the cortex, glutaminase converts glutamine to glutamate and ammonia, the latter then being excreted in the urine. The glutamate is further deaminated, yielding more ammonia to the urine, and the resulting α -ketoglutarate goes by associated reactions to oxaloacetate and then by gluconeogenesis to glucose 6-phosphate. Glucose 6-phosphate formed by phosphorylation of glucose in the renal medulla is a zonated pool separate from glucose 6-phosphate formed by gluconeogenesis in the cortex. One should expect that the regulation of glycolysis, suppressing it in the cortex and stimulating it in the medulla, and regulation of gluconeogenesis, suppressing it in the medulla and stimulating it in the cortex, might be reflected by the production and disposition of fructose 2,6-bisphosphate in those regions. Some work (316) has been performed showing that both cortex and medulla contain that effector as well as the enzymes required in its synthesis and degradation. Some evidence has been obtained that differences exist between the kidney enzyme and the liver enzyme, as might be expected.

CONCLUSION

Any review of the biochemistry and physiology of the liver that attempts a coherent synthesis of available facts is hindered because the relevant information has been obtained from studies performed with several different animal species and use of different kinds of preparations. Most investigations have been performed in rodents, especially the rat, although chicks, dogs, and humans also have been used. The preparations employed have been isolated perfused livers, liver homogenates, liver slices, and suspensions of hepatocytes. Obviously, the use of any of those preparations removes the tissue from highly important interactions with other organs and, in many instances, provides only a description of existing pathways of metabolism and an evaluation of the potential capacities of these pathways. Also, since the metabolism of the liver greatly depends on minute-to-minute changes in the activity and metabolism of the whole organism, the limitations imposed by use of isolated organs, tissues, or cells are severe. Furthermore, findings in one species may not apply to others, as would seem to be evident in the matter of conversion of carbohydrate to fat in humans as opposed to other species.

The difficulties and limitations noted above were illustrated in two studies using hepatocytes isolated, respectively, from an avian source, the Japanese quail (317), and from the rat (208). In the former study, although the quail had been fed, the hepatocytes obtained after perfusion of the liver were low in glycogen contents and were almost depleted of alanine and glutamine. Incubation of the cells with a medium containing alanine caused remarkable increase in glucose uptake, glucose oxidation, and conversion of glucose carbon to fatty acids. That was in sharp contrast to the well-documented findings in the rat that, under conditions of lipogenesis after a carbohydrate meal, hepatocytes incorporate little of glucose carbon into fatty acids; that is, they show limited utilization of glucose. In the second study to which we refer, hepatocytes, isolated from starved rats, and incubated with suitably labeled glucose, showed that about 50% of the hexose had been first degraded to 3-carbon compounds before incorporation into glycogen. However, hepatocytes obtained from fed animals incorporated glucose directly into glycogen without prior conversion to 3-carbon units. The difficulty of extrapolating these observations to the whole animal is apparent; the experiments show a potential but do not reveal how it would be modified by whole-body hormonal and substrate influences.

This chapter has attempted to show how the liver obtains energy to conduct a large number of functions related to transport, biosynthesis of macromolecules and special small molecules, and biotransformation of metabolites and drugs. Many syntheses are performed in service to other organs or tissues, for example, the manufacture of plasma proteins, carnitine, and creatine. On the other hand, the liver collects and transforms substrates to meet the specific fuel requirements of other tissues in response to metabolic signals coming from the whole organism. Remarkably, the liver is probably the only organ that produces acetoacetate for use by muscle, brain, and kidney cortex, yet it does not use that substrate significantly for its own energy metabolism. The liver is also the major organ for production of glucose by gluconeogenesis and yet uses little glucose for its own metabolic requirements. The liver expends energy in the storage of glycogen, which subsequently is degraded to yield glucose for other tissues, such as the brain and erythrocytes, while it again uses little of that glucose for its own requirements. The liver can make fatty acids for export as triacylglycerols and phospholipids but uses fatty acids originating from the diet and the adipose tissue for its own energy needs.

The liver does not have much capacity for transamination of leucine, isoleucine, and valine but can actively oxidize the corresponding branched-chain α -keto acids arising from transamination in the muscle. It has special capacity for conducting gluconeogenesis from alanine arising in muscle, where the amino groups of the branched-chain amino acids are transferred to pyruvate by transamination.

All these energy-related functions of the liver are accomplished in ways that are highly regulated by hormones and other agonists, especially by glucagon and insulin. The substrates coming from the liver, such as glucose and acetoacetate, in turn may be primary signals to other tissues to activate or inhibit pathways in the utilization of one or another fuel. Thus, acetoacetate produced in the liver may signal the adipose tissue to reduce the extent of lipolysis that provides fatty acids to the liver for production of ketone bodies; and it may signal the muscle to limit proteolysis and activate the pathways for use of ketone bodies for energy. The liver can be said to sense the fuel needs of all of the other tissues in the body and respond by adjusting its metabolism accordingly.

The contribution of the energy metabolism of the liver to the maintenance of acid-base balance in the liver has been explored only tentatively. The production of glucose, a neutral molecule, from lactate and pyruvate is an energy-consuming process that also removes protons. The kidney, also a glucose-producing organ, probably uses the gluconeogenic pathway not only for facilitating ammonia production but for taking up protons. The possible function of the urea cycle in acid-base balance is the subject of an interesting and provocative review (318).

One cannot doubt that current descriptive knowledge of the metabolic functions of the liver is profound, and something is known of the integration of pathways. However, our understanding of the dynamic, rapid responses of the liver to changes in the physiologic condition of the total organism is only beginning to be obtained.

ABBREVIATIONS AND DESIGNATIONS

Abbreviations and designations used in this chapter are as follows: Compounds: F6P (fructose 6-phosphate); F1,6-P₂ (fructose 1,6-bisphosphate = fructose 1,6-diphosphate); F2,6-P₂ (fructose 2,6-bisphosphate); LAC (lactate); PYR (pyruvate); PEP (phosphoenolpyruvate); OAA (oxaloacetate). Enzymes: 6PF-1K (6-phosphofructokinase-1 = 6-phosphofructokinase, the enzyme that converts F6P to F1,6-P₂); 6PF-2K (6-phosphofructokinase-2, the enzyme that converts F6P to F2,6-P₂); F1,6-Pase or Fru 1,6-P₂ase (fructose 1,6-bisphosphatase, the enzyme that converts F1,6-P₂ to F6P + P_i); F2,6-P₂ase or Fru 2,6-P₂ase (fructose-2,6-bisphosphatase, the enzyme that converts F2,6-P₂ to F6P + P_i); PK (pyruvate kinase).

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