mainly in the form of bile acids. Specific activity of the excreted bile acids was 2.5 times higher in EE-treated rats than in untreated controls.
Similar results were obtained using liposomes as artificial vehicles for ^{3}H-CO. Thus, large multilamellar vesicles (MLV), composed of cholesterol, sphingomyelin, phosphatidylserine and ^{3}H-CO in a 49½:40:10:½ molar ratio, were cleared very rapidly by Kupffer cells ($t_{½}$~1 min). After 3 hours only 1.9±0.2% of the injected radioactivity had appeared in bile. Small unilamellar vesicles (SUV; cholesterol: phosphatidylcholine: phosphatidylserine: ^{3}H-CO, 49½:40:10:½) were directed predominantly to hepatocytes ($t_{½}$~10 min) and the 3 hours biliary recovery was 6.5±1.2%. Again, biliary radioactivity was mainly in the form of bile acids. SUV-derived radioactivity was found markedly enriched in the muricholic acid fraction; MLV-Co was mainly converted to cholic acid.
In conclusion: newly synthetized hepatic cholesterol is quantitatively the main source for bile acid synthesis in the rat, although a specific bile acid, β-muricholic acid, is highly dependent on pre-existing cholesterol. Uptake of CO by hepatocytes is followed by a relatively efficient excretion of its derivatives, i.e. bile acids, into bile. Kupffer cell uptake is less efficiently coupled to biliary excretion, and probably depends on the rate of cholesterol transport from Kupffer cell to hepatocyte.

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Acknowledgement:
This work is supported by the Dutch Heart Foundation.

KETOGENESIS AND CARBOHYDRATE AVAILABILITY

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It is now well-established that ketone bodies are important alternative substrates to glucose in mammalian tissues, in particular the brain (1). Under normal conditions, as the availability of glucose decreases (e.g. during fasting) the level of ketone bodies in the circulation increases and their utilization , which is concentration-dependent, is enhanced. When carbohydrate is made available again, the concentration of ketone bodies rapidly falls. The key question is how this reciprocal relationship between these respiratory substrates is achieved? The available evidence indicates that it is brought about by changes in concentration of plasma hormones which in turn alter metabolism in adipose tissue and liver (2). As the blood glucose decreases there is a concomitant decrease in plasma insulin which in turn results in a stimulation of lipolysis in adipose tissue. One of the most potent effects of insulin being its antilipolytic action in this tissue. Increases in plasma glucagon or catecholamines (e.g. as a consequence of hypoglycaemia) relative to the prevailing insulin concentration will also increase lipolysis and flux of fatty acids from adipose tissue.
Long-chain fatty acids derived from adipose tissue are the major precursors of ketone bodies and therefore whenever the flux to the liver increases the rate of ketogenesis might be expected to increase. However, the fate of fatty acids within the liver is finely regulated by the hepatic carbohydrate availability as well as to external signals (insulin versus glucagon) (2). Thus long-chain fatty acids can either be esterified and secreted as VLDL (high carbohydrate state) or enter the mitochondria for oxidation to ketone bodies or CO_2 (low carbohydrate state).
The integration of fatty acid and carbohydrate metabolism in the liver is brought about by changes in the concentration of malonyl-CoA, an intermediate in the de novo synthesis of fatty acids from glucose (or lactate) (2). This metabolite inhibits the activity of carnitine acyltransferase I (CAT I) which initiates the entry of long-chain fatty acids into the β-oxidation pathway. Insulin can increase the concentration of malonyl-CoA by activation of the enzyme, acetyl-CoA carboxylase, which controls its synthesis.
Glucagon has the opposite effect. Recent studies indicate that CAT I is less sensitive to malonyl-CoA inhibition in insulin-deficiency or fat feeding, which are associated with increased ketogenesis. Glucagon can also activate CAT I by covalent modification (phosphorylation) (3) and it is possible that this may be responsible for the decrease in sensitivity to malonyl-CoA.
This overview of the integration of ketogenesis and carbohydrate availability will be discussed in relation to two inborn errors of hepatic carbohydrate metabolism, namely glycogen synthetase deficiency (4) and glucose-6-phosphatase deficiency (5) which are associated with hypoglycaemia but widely different concentrations of blood ketone bodies.

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THE GLUCOSE PARADODX: IS GLUCOSE THE PRECURSOR OF LIVER GLYCOGEN?

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The consumption of carbohydrate after a fast is followed by glycogen deposition in the liver, and carbohydrate feeding induces hepatic lipogenesis. Common sense suggests that glucose is the precursor for glycogen and fat; and indeed the direct conversion of glucose into glycogen via hexose phosphate and UDP glucose is depicted in most textbooks. However, a large body of experiments shows conclusively that a major part of glycogen is not directly derived from glucose but from 3 carbon precursors formed by glucose cleavage. Such a pathway appears energetically wasteful, and hence has been designated the "glucose paradox". I present here the experimental evidence for the occurrence of the indirect pathway, and I dwell upon the unsolved problems in the area. Relevant literature references are found in two recent reviews (1,2).
The evidence in vivo and in vitro for the limited capacity of liver to take up glucose even in the presence of a substantial glucose load and to convert it directly to glycogen is as follows: a) Isolated perfused liver and hepatocytes have a high capacity for gluconeogenesis, but show very little net uptake of glucose at physiological concentrations (below 15 mM), and form virtually no glycogen when

glucose is the sole substrate. When supplemented with 3 carbon compounds such as lactate, glycerol, alanine, there occurs a simultaneous formation of glucose and glycogen at the expense of the 3 carbon substrates; b) lactate is a much better precursor than glucose for hepatic lipogenesis in vivo and in vitro; c) in animals and in man fructose (which is metabolized via triose phosphate) is a better precursor of hepatic glycogen than is glucose; d) after the administration of glucose by mouth or intravenously, it is difficult to demonstrate in animals and in man net uptake of glucose by portal-hepatic vein analysis. Net glucose uptake occurs in animals conditioned to a high carbohydrate diet or upon administration of heavy glucose loads in the presence of a low glucagon/insulin ratio, but the uptake of glucose is substantially less than the deposition of glycogen; e) after administration of U-^{14}C glucose, the specific activity of glycogen glucose is less than that in the circulation, indicating production of glycogen from non-glucose carbon; f) when rats are injected with glucose labelled with ^{14}C in carbon 1 or 6, an extensive scrambling of ^{14}C in the glucose carbon skeleton occurs. When glucose is labelled with ^{14}C and tritium there is a preferential loss of tritium relative to ^{14}C in glycogen. These findings indicate a cleavage of glucose to a 3-carbon compound prior to conversion to glycogen; g) when glucose and ^{3}HOH are administered, there is incorporation of tritium on carbon 1 and 6 of liver glycogen, which must occur in the synthesis of glucose from pyruvate.

There is presently controversy as to the relative contribution of the direct and indirect pathway to glycogen synthesis. The majority of studies is that in rats, half or more of liver glycogen is formed indirectly. The effect of hormonal and dietary conditions, pathways of glucose administration (intragastric, intraportal, intravenous) and of species differences remains to be investigated.

A possible reason for the limited capacity of liver to utilize glucose is the relatively low rate of hepatic glucokinase, combined with the concurrent activity of glucose-6-phosphatase, causing futile cycling and hydrolysis of glucose 6P back to glucose. So far no effectors of the rate of glucokinase and glucose-6-phosphatase have been found.

Several unresolved problems remain: a) The hepatic uptake of the lactate or other 3-carbon compounds appears inadequate to account for the synthesis of glycogen by the indirect path. The site of formation of such putative 3-carbon precursors is obscure; b) the synthesis of hepatic glycogen by the indirect path occurs in the presence of high levels of fructose-2,6P. This compound is known to activate phospho fructokinase and inhibits fructose-6-phosphatase, thus presumably blocking synthesis of hexose from triose phosphates.

To resolve the questions it has been proposed that gluconeogenesis and glycogen synthesis by the indirect path occurs predominantly in the cells of the periportal zone of the liver, whereas glycolysis and glycogen synthesis from glucose occurs in cells of the perivenous zone of the liver. According to this concept the metabolic pattern of liver metabolism is heterogenous, and in effect different or even opposing reactions and processes occur simultaneously in different zones of the liver. Current studies in our laboratory on the distribution of enzymes and fructose-2,6P through the liver will be described.

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THE UTILIZATION OF SUBSTRATES AND O_2 BY THE MAMMALIAN FETUS: CHANGES DURING DEVELOPMENT

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In the past two decades a great deal of information has been acquired with respect to the metabolism of the mammalian fetus. For the better part, the data have been collected in chronically catheterized animals, representative of steady state conditions. Thus, meaningful interspecies comparisons have become possible. The species studied include the horse, cow, pig, goat, guinea pig and rabbit. But by far the most data have been collected in the sheep.

Several features of fetal metabolism have been well established by these studies. Among these are: a relatively high rate of fetal O_2 consumption, which, when viewed across species, appears relatively constant on a per kg basis, in the order of 8 ml/kg/min. In all species, the placenta has a high rate of lactate production under aerobic conditions, and the lactate is consumed by the fetus. A similar pattern has been shown for placental production and fetal consumption of NH_3. In the fetal lamb aminoacids transported across the placenta provide the bulk of the carbon and nitrogen required by the fetus.

More recently, the utilization of nutrients within the fetus has been studied. Two different lines of investigation have been pursued. First, individual organ uptakes and release of substrates and O_2 have been studied. In the fetal lamb, data are now available for brain, heart, kidney, GI tract, liver and hind limb, all studied under chronic steady state conditions. Secondly, tracer methodology has been used to investigate the utilization of aminoacids and carbohydrates by the fetus (1).

However, until recently, all such studies of fetal metabolism had been carried out in the latter part of gestation. In man, the advent of techniques permitting us to obtain fetal blood samples at any stage of gestation from 17 weeks to term, has made it imperative to acquire knowledge of the biology of the early or mid gestation fetus. We have now been able to catheterize the mid gestation fetal lamb permitting studies under steady state conditions, when the fetus is only approximately 200 gms. These studies have shown a higher O_2 saturation and lower hemoglobin concentration in the fetus, coupled with a much higher metabolic rate, when expressed on a dry weight basis (2,3). Fetal rates of protein synthesis and leucine oxidation have been studied using (14C) leucine. The protein synthetic rate is higher than at term, but, expressed as a fraction of O_2 consumption, is unchanged from late gestation (4).

The increased use of fetal blood sampling in man for diagnosis of congenital infections or genetic disorders in mid pregnancy as well as for assessment of fetal well-being in late pregnancy has provided an opportunity to obtain a similar description of the fetal environment in man from mid to late gestation. Studies conducted jointly by the University of Colorado and the University of Milano have provided a description of some of the changes in oxygenation and acid base balance as well as transplacental glucose gradients of the latter half of gestation in man. The data from these studies are now being organized for publication.