of fructose bisphosphatase, glucose 6-phosphatase or phosphoenolpyruvate carboxy-kinase when expressed per g wet wt. of liver, but they significantly increased the liver weight of the embryos and hence the total gluconeogenic capacity of the individual embryo (R. S. Campbell & D. R. Langslow, unpublished observations). Hence several different hormones can modify the activities of gluconeogenic enzymes in chick-embryo liver.

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Gluconeogenesis in Ruminants
D. B. LINDSAY

Department of Biochemistry, A.R.C. Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, U.K.

Ruminants are characterized by having their gluconeogenic pathway more or less permanently switched on. Indeed the rate of gluconeogenesis is greatest after a meal, and slowly declines during starvation. There are several reasons for believing that little dietary glucose is available. (1) Most of the dietary carbohydrate can be accounted for by the amounts of volatile fatty acids, methane and CO₂ produced
through fermentation in the rumen. (2) Although starch may escape fermentation when certain grain diets are fed, the maltase activity in the small intestine (Coombe & Siddons, 1973) may be insufficient for rapid hydrolysis of glucose. Absorption of glucose from the small intestine, however, can only occur from fairly high concentrations of glucose, since active transport, at least from the sheep small intestine, is rather rudimentary (Scharer, 1976). (3) Blood glucose does not increase significantly after a meal. Although Symonds & Baird (1976) have demonstrated an increase in mesenteric-vein glucose in cows on a grain diet, calculations suggest that the amount of glucose absorbed will be less than 10% of the circulating glucose. Thus in nearly all circumstances, 90–100% of the circulating glucose is derived from gluconeogenesis.

Gluconeogenic precursors

The post-prandial gluconeogenesis of ruminants has a different pattern of substrate utilization from that of starved animals. Most of the studies have been made with whole animals, usually by infusing a 14C-labelled precursor either intravenously or intraruminally. Either at equilibrium or over some suitably long time, comparison is made of the specific radioactivity of precursor and of plasma glucose. A major problem with this technique was pointed out some years ago (Krebs et al., 1966). Because most of the glucogenic precursors pass through, or are in rapid equilibrium with, reactions of the tricarboxylic acid cycle, 14C from such compounds may appear in CO2 rather than glucose. This crossover leads to an under-estimation of the gluconeogenic potential of a precursor. Correction can be made for this by determining the fraction of the flow into oxaloacetate that passes to phosphoenolpyruvate (Thompson, 1971; Vinay et al., 1978). This fraction can be estimated from the specific radioactivity of the individual carbons of glucose after infusion of specifically labelled precursors, and on this basis (from studies with specifically labelled propionate or acetate), I estimate that the technique might result in values that are 15–20% low. There are a number of assumptions underlying such calculations and it seems preferable to assess the reliability of uncorrected estimates by comparing them with the maximal contribution, obtained as the ratio of the net hepatic uptake of precursor to the glucose output or isotopically determined glucose flux. Such results are shown in Table 1. It should be borne in mind that for few of these results was comparison made in the same animal.

Propionic acid is the most important precursor; moreover there is reasonable agreement between the two estimates, which perhaps suggests that the correction for crossover

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>(a) Isotope dilution</th>
<th>(b) Hepatic uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propionate</td>
<td>0.42 ± 0.056 (4)</td>
<td>0.41 (2)</td>
</tr>
<tr>
<td>Isobutyrate + valerate</td>
<td>—</td>
<td>0.05 (1)</td>
</tr>
<tr>
<td>Lactate</td>
<td>0.19 ± 0.064 (3)</td>
<td>0.22 ± 0.068 (3)</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0.047 (1)</td>
<td>0.04 (2)</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.05 (2)</td>
<td>0.07 ± 0.020 (3)</td>
</tr>
<tr>
<td>Glutamine</td>
<td>0.05 (2)</td>
<td>0.05 (2)</td>
</tr>
<tr>
<td>Serine</td>
<td>0.008 (1)</td>
<td>0.02 (2)</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.01 (1)</td>
<td>0.05 (2)</td>
</tr>
<tr>
<td>Aspartate</td>
<td>0.005 (1)</td>
<td>0.01 (2)</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.006 (2)</td>
<td>0.015 (2)</td>
</tr>
</tbody>
</table>

Table 1. Fraction of glucose synthesized by various metabolites in fed mature sheep

In (a) estimation was by infusion of 14C-labelled substrate and comparison of specific radioactivities of glucose and substrate. In (b) estimation was by comparison of the ratio of hepatic uptake of glucose (or glucose turnover as estimated by isotope dilution) to hepatic uptake of substrate. Values are taken from a number of publications (see Lindsay, 1979) and some unpublished work. Values in parentheses indicate the numbers of studies.
may not be as important in vivo as is usually supposed. For the other glucogenic fermentation acids (isobutyric and valeric) the possible contribution, although small, is not negligible. Lactate and glycerol are endogenous sources and in assessing their significance account should also be taken of renal gluconeogenesis. About 10% of glucose synthesized is derived from the kidney in sheep (Kaufman & Bergman, 1971, 1974) of which about 65% may be accounted for by uptake of lactate and pyruvate and about 12% by glycerol. Thus the total contribution of lactate to glucose synthesis, as assessed by renal and hepatic uptake, is about 28%, and for glycerol about 5%. There is thus probably some correction for crossover for lactate. Glycerol should not require such a correction and the results support this.

For the amino acids, interpretation of results by the isotope-dilution technique is further complicated by the release of endogenous amino acids in gluconeogenic tissues. Alanine is also taken up by the kidney (Bergman et al., 1974) and on the basis of hepatic plus renal uptake it may account for about 9% of glucose output, a value that is appreciably greater than the estimate by isotope dilution. Glutamine is released by the kidney in fed sheep and the two estimates of gluconeogenic potential shown in Table 1 are in reasonable agreement. Unlike the rat, the sheep uses little serine for glucose production and glycine is also probably a poor gluconeogenic source (the substantial hepatic uptake shown may reflect a requirement for hippuric acid synthesis). The pathway for conversion of serine and glycine into glucose is probably different in the sheep compared with the rat. In studying the transfer of [14C]formate to serine in sheep, Annison & White (1962) found the activity largely in carbon 3 and in glucose in carbons 1 and 6. These results suggest that serine (formed from glycine and formate) is incorporated into glucose through hydroxypyruvate and not pyruvate (which would lead to activity in glucose carbons 1, 2, 5 and 6). Threonine may follow a similar pathway in sheep. We have found, after infusion of [U-14C]threonine in phlorrhizin-treated sheep, that the specific radioactivity of carbon 6 of urinary glucose is only about one-fifth of the mean glucose specific radioactivity. If threonine passes through propionyl-CoA (the pathway usually assumed), the activities in carbons 1, 2, 5 and 6 of glucose should be greater than in carbons 3 and 4 because of the introduction of non-radioactive CO2 in the carboxylation reaction leading to succinate. A pathway involving threonine aldolase would lead to the formation of acetyl-CoA and glycine. Conversion of the glycine to serine and then glucose would then result in a lower radioactivity in glucose carbons 1 and 6.

Control of gluconeogenesis

It is possible to produce isolated hepatocytes from sheep liver (Clark et al., 1976; Ash & Pogson, 1977; Morton et al., 1976). Such preparations do have some abnormalities. Oxygen consumption, in the absence of added substrate (Clark et al., 1976), is much higher than in the liver perfused without substrate (Linzell et al., 1971); but the increase when substrate (lactate) was added was less than in the perfused liver (when propionate or amino acids were added) (Lindsay et al., 1975). Gluconeogenesis in response to some substrates (glycerol, amino acids) is poor, but the response to propionate is better than that of the perfused liver. Finally, there is some hormonal sensitivity since gluconeogenesis can be increased by 1 nm-glucagon.

Studies with whole sheep suggest that the major factor determining the rate of gluconeogenesis from a substrate is its inflow (see Fig. 1). For lactate the effect of changing flux on glucose synthesis from lactate is marginal (P = 0.06); in addition the response is substantially less at a given flux than that of propionate or glyceraldehyde 3-phosphate. Within the range of flux shown, there was not much effect on total glucose output, but at higher inflows glucose output increases both for propionate (Judson & Leng, 1973) and for glyceraldehyde 3-phosphate (A. Ranaweena & E. J. H. Ford, personal communication). Infusion of casein hydrolysate also increases glucose output (Lindsay & Williams, 1971; Lindsay & Dyke, 1974), the increase being related to the rate of infusion (Judson & Leng, 1973).

The limited response to change in lactate input is supported by results obtained by Watson (1972) and myself (unpublished results). In sheep receiving a range of diets,
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Fig. 1. Dependence of rate of glucose synthesis from precursor on whole-body turnover of precursor

o, Glycerol (Bergman et al., 1968); △, propionate (Judson & Leng, 1973); □, lactate (Annison et al., 1963; Prior & Christenson, 1977).

measurements were made of both gluconeogenic enzyme activities and glucose-production rates. There was a significant relation between glucose-production rate and either total fructose 1,6-bisphosphatase or total phosphoenolpyruvate carboxykinase activity. Total pyruvate carboxylase activity, however, did not vary with diet.

Activators

Clark et al. (1976) found, using lamb hepatocytes, a marked stimulation of gluconeogenesis by butyrate (10–50μM). Judson & Leng (1973), however, were unable to obtain any consistent stimulation of gluconeogenesis from propionate after intraportal infusion of butyrate. This could be because the butyrate concentration in portal blood was already about 50μM before butyrate was infused; or perhaps because the effect of butyrate was on pyruvate carboxylase.

Glucagon stimulates gluconeogenesis and glycogenolysis in both isolated hepatocytes (Clark et al., 1976) and the whole animal (Brockman & Bergman, 1975; Brockman et al., 1975), where there is increased uptake of glycerol, lactate and amino acids.

Inhibitors

Glucose is an effective suppressor of gluconeogenesis in starved sheep (Annison & White, 1961). The inhibition of gluconeogenesis, although related to the amount of glucose infused, is less than complete in fed sheep (Judson & Leng, 1973) and cattle (Thompson et al., 1975). It seems (Judson & Leng, 1973) that gluconeogenesis from propionate is less inhibited than gluconeogenesis from endogenous sources. Glucose is unlikely to have a direct effect on the output of glucose by the liver, but is more likely to act by increasing insulin secretion. Clark et al. (1976) were unable to demonstrate a direct effect of insulin in inhibiting gluconeogenesis from lactate in lamb hepatocytes, but insulin did appear to oppose the action of glucagon, and insulin infused into the portal vein of sheep lowered glucose output (West & Passey, 1967). It may be relevant that insulin secretion in ruminants is stimulated not only by glucose but also by several volatile fatty acids (propionate, butyrate, isovalerate and valerate) (Horino et al., 1968).

Ruminants require a capacity for high rates of gluconeogenesis in periods dominated by anabolic reactions (pregnancy and lactation) yet as in other animals the flow must be controlled during starvation. One might speculate that this is determined by arranging matters so that the flow of dietary precursors (propionate and valerate) through the gluconeogenic pathway is relatively unrestrained. Only when the capacity of the liver to remove propionate or valerate is exceeded is control asserted (through stimulation of insulin secretion). Control by factors other than insulin would be directed to the pyruvate
carboxylase reaction, and in control at this site one need not be surprised to find features in common with those in non-ruminants.

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