Conference: Hepatic Metabolism of Organic Acids in Ruminants

Ruminant Hepatic Metabolism of Volatile Fatty Acids, Lactate and Pyruvate[1]

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ABSTRACT Ruminant liver has a quantitatively unique array of substrates presented to it because of the extensive fermentation of dietary carbohydrate to organic acids in the gastrointestinal tract. The single largest input of dietary energy to the extrasplanchnic tissues is acetic acid derived from fermentation, which is largely unused by hepatic parenchyma. The other volatile fatty acids derived from fermentation, primarily propionate, are cleared extensively, but not completely, by the liver. This results in a marked concentration gradient for these acids across the liver lobule. L-lactate, derived from tissue metabolism, as well as variable amounts from rumen fermentation, is used by the liver at a rate lower than for propionate and below the predicted capacity based on in vitro enzymatic and intact cell capacity data. The net result of this selective utilization by the liver results in peripheral blood containing significant concentrations of L-lactate and acetate, but little of the other organic acids. Propionate carbon metabolized by liver cells is converted to glucose with little true loss of carbon, but the same is not true of lactate carbon. The energetic efficiencies by which propionate and lactate carbon are converted to glucose may be much less than optimal because of extensive cycling through pyruvate kinase, pyruvate carboxylase and phosphoenolpyruvate carboxykinase. Inhibition of this futile cycling may represent one avenue by which energetic costs of maintenance and production can be lowered in ruminants. J. Nutr. 122: 838–842, 1992.

INDEXING KEY WORDS:

- *ruminants* • *hepatic* • *gluconeogenesis*
- *organic acid*

The major purpose of this paper is to review relevant data about hepatic metabolism that has been obtained in the last decade, to integrate them with previous data and to identify important questions and opportunities related to domestic ruminants. Duplication with the most recent reviews of hepatic metabolism of propionate (1), both lactate isomers (2) and other aspects of splanchnic glucose and energy metabolism (3) has been avoided.

The primary techniques used in this research had been applied to the study of ruminant liver function before 1980, with the exception of hepatocyte monolayer cultures, which have been applied only recently to cattle and sheep (4–6). The latter technique has recently proved valuable in providing an in vitro system responsive to protein hormones at physiological concentrations, thereby allowing the direct effects of insulin and glucagon on hepatic metabolism of specific substrates to be determined (7). Monolayer cultures should also be useful for studying processes that require incubations of more than a few hours and processes that may be strongly influenced by the instabilities inherent in freshly isolated hepatocytes. Diligent application, refinement and improvement of existing in vivo techniques, including isotope dilution, *trans*-organ balance and their combination, has provided new information on in vivo metabolic rates in both cattle and sheep. Although this review focuses on hepatic metabolism, the interplay of the gut and liver as an integrated splanchnic unit providing nutrients to extrasplanchnic tissues should not be ignored.

Net portal appearance (NPA[3]) of a substance is the concentration difference between arterial and portal blood multiplied by portal blood flow. This measure-

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[3] Net portal absorption (NPA), pyruvate dehydrogenase (PDH), volatile fatty acids (VFA).

ment reflects the net contribution of the gastrointestinal tract to the liver and extrasplanchnic tissues. It includes total absorption from the lumen, plus any production (as for L-lactate) by the gastrointestinal tissues, minus metabolism by these same tissues of the substance derived from either the arterial blood or the lumen. Acetate and propionate account for the majority of the NPA of energy in sheep fed 800 g of alfalfa/d (68 and 18 mmol/h) (8) and in lactating dairy cattle fed 178 MJ/d of metabolizable energy (1880 and 790 mmol/h or 40% of metabolizable energy intake) (9). Actual contribution of acetate from the gastrointestinal lumen to turnover of blood acetate, as measured by isotope dilution, will be greater than NPA because of metabolism of blood acetate by the gut (8), and such metabolism is probably much less for the other volatile fatty acids (VFAs) that occur at low arterial concentration.

Acetate is poorly oxidized by liver cells in vitro (10), and liver has no mitochondrial, and only limited cytosolic, acetyl-CoA synthase activity (11, 12). Despite this, in vivo *trans*-hepatic measurements indicate some uptake of labeled acetate in sheep (8, 13). This minor amount of utilization may be for biosynthetic, rather than oxidative, purposes or may represent uptake by nonparenchymal cells. In any event, acetate production by liver usually exceeds any possible utilization, so that the liver is a net producer of acetate (8, 9). Hepatic acetate production is presumed to function as a means of converting long-chain fatty acid energy into a water-soluble form, thereby mimicking ketogenesis. However, although starvation increases production of ketones, it decreases net hepatic production of acetate (14).

Net hepatic uptake of other VFAs reflects total metabolism because these compounds presumably are not produced by mammalian tissues. Hepatic clearance of propionate is extensive, accounting for 85% of propionate flow to liver and 93% of propionate net portal appearance (9). The former clearance results in low peripheral arterial concentration, whereas the latter indicates that only 7% of propionate NPA is used by extrasplanchnic tissues. Clearance of *n*-butyrate is somewhat lower, 68% of total butyrate flow to liver and 84% of butyrate NPA (9). In vitro measurements indicate that hepatocytes have over twice the capacity for propionate uptake as for butyrate (15). This comparison suggests that the liver contains considerable reserve capacity to clear propionate in vivo.

The effect of propionate concentration on propionate uptake and metabolism to glucose and CO_2 has been determined in vitro in hepatocytes (7, 16, 17). In vitro saturation is reached at concentrations between 2 and 5 mmol/L. As in vivo concentrations of portal blood are usually <2 mmol/L, even the periportal hepatocytes are probably not functioning at maximum capacity to metabolize propionate. These kinetic data further support the concept that the liver has reserve capacity to metabolize propionate. However, on high barley diets there is apparently increased incorporation of propionate carbon into fat depots due to high propionate production (18). Also, the capacity for propionate metabolism can be reduced during fatty infiltration of the liver caused by negative energy balance (19).

Ruminal production and NPA of the branched VFA and valerate are minor in terms of energetic importance and perform no known unique function. In vivo hepatic clearance of isobutyrate and 2-methyl butyrate are similar to that for propionate and valerate (9). Hepatic removal of valerate is similar to that for propionate both on a percentage basis in vivo and total uptake at 1.25 mmol/L in vitro (9, 15). Uptake of isovalerate by hepatocytes in vitro exceeded that of butyrate, each at 1.25 mmol/L, but clearance in vivo was highly variable, perhaps because of the very low concentrations of these minor VFAs in both portal and arterial blood (9, 15). As these minor VFAs seldom exceed 0.05 mmol portal blood/L, it is unlikely that increased ruminal production would lead to hepatic saturation in vivo.

L-lactate flow to the liver is roughly equivalent to propionate flow, but much of this flow represents a continuously circulating pool of lactate and production by extrasplanchnic tissues. In lactating cows, L-lactate delivery to the liver is 765 mmol/h, with 28% of this derived from NPA (20); in pregnant sheep these values were 104 mmol/h and 8%, respectively, and in lactating sheep, 173 mmol/h and 9%, respectively (21). Hepatic clearance (% of total lactate flow to liver) was 33% in lactating cows, 28% in pregnant sheep and 12% in lactating sheep. Propionate and L-lactate total flow to liver, and therefore average portal plus arterial concentration, are similar in vivo, yet uptake of lactate is less than half that for propionate, even though propionate uptake appears to be well below capacity.

Net hepatic uptake of pyruvate is negligible in fed cattle and low but measurable in starved cattle, with pyruvate uptake seldom exceeding 10% of L-lactate uptake (14). Ruminant liver can be supplied with D-lactate produced by microbes in the gastrointestinal lumen, and D-lactate is metabolized to glucose and CO_2 at half the rate of L-lactate in vitro (2).

In vitro metabolism by plated bovine calf hepatocytes indicate a capacity for L-[U-^{14}C]lactate conversion to glucose approximately half that for [2-^{14}C]propionate over physiological and supraphysiological concentrations of each substrate (7). Previous experiments in our lab with freshly isolated goat hepatocytes suspended in Krebs-Ringer buffer (22) indicated lactate conversion to glucose was <10% of propionate conversion to glucose (7 and 85 nmol $\cdot$ h^{-1} $\cdot$ mg dry cell^{-1}). In these earlier experiments, the utilization of lactate was increased, and propionate decreased, when NH_4Cl was added to the media (22), resulting in a ratio approaching that found in plated bovine hepatocytes. Short-term exposure of plated bovine hepatocytes to

physiological concentrations of insulin reduced propionate, but not lactate, conversion to glucose, but even in the presence of insulin, lactate was converted to glucose at a lower rate than propionate (7). These comparisons suggest that lactate metabolism is a function of undefined regulators maintaining lactate metabolism below maximum capacity in vivo, that at least some of the factors that limit lactate utilization do not limit propionate utilization and that the specific gluconeogenic rates inhibited by insulin are not the rate-limiting steps for lactate in vitro.

Ruminants show little NPA of glucose (20), and therefore hepatic gluconeogenesis provides the glucose needed for maintenance, support of the fetus and synthesis of milk lactose. An examination of rumen propionate production versus blood glucose irreversible loss (1) suggests that propionate is produced just at or below a rate sufficient to provide the sole source of carbon to meet glucose demands, but because 33% of rumen propionate is metabolized during absorption (23), other sources of glucose carbon must exist. When liver uptake of propionate and lactate is compared with glucose output, they may account for a maximum of 55% and 17% of glucose output, respectively, assuming complete conversion to glucose (9). Therefore, it is apparent that ruminants, especially lactating dairy cattle and goats, and pregnant sheep and goats, have a real need to effectively transfer carbon from glucose precursors into glucose. The energetic efficiency with which this process is conducted is a separate matter, which can affect both the maintenance energy requirements of the animal and the partial efficiency of production.

Carbon transfer, as measured by isotope incorporation, is underestimated by exchange of labeled carbon for unlabeled carbon in processes that cause no net loss of carbon for gluconeogenesis, such as oxaloacetate cycling through the tricarboxylic acid cycle. Conversely, carbon transfer is overestimated due to the incorporation of any nonglucogenic carbon, usually from acetyl-CoA or CO_2, which may arise from the labeled substrate and then be incorporated into glucose. The underestimation appears to be the dominant error when measuring the conversion of propionate carbon into glucose. Only one experiment has used adequate correction for these effects in vivo, and this experiment contains complete data for only one sheep (24). This work suggests that over 95% of propionate appearing in the mesenteric vein is converted into glucose. This approximates the hepatic removal of propionate, indicating that 100% of hepatic propionate uptake is metabolized to glucose. Failure to correct for label dilution in this data set would result in an estimate of 63%, rather than 95%, of propionate converted to glucose. Most experiments calculate a transfer quotient, the fraction of glucose arising from propionate, and this value is 54% in reference 24. This number is actually smaller than the mean of 43% of glucose from ruminal propionate calculated from four steers (25) once the former is adjusted downward to correct for the 33% loss of propionate carbon during metabolism by the nonglucogenic gut tissues (0.67 × 54% = 36%, compared with 43% in steers) and is similar to values summarized in reference 26. Also, the increased incorporation of labeled carbon from propionate into glucose after exogenous propionate infusion was similar in these two experiments (24, 25). It is clear that the techniques suggested in reference 24 must be applied in more animals; however, the data from this paper are supported by other isotope dilution data sets where comparisons can be made.

In addition to showing complete efficiency of propionate carbon use for glucose, the work of Steinhour and Bauman (24) indicates that ~50% of total oxaloacetate turnover is cycled through phosphoenol-pyruvate, pyruvate and returns to oxaloacetate, while ~30% cycles through the tricarboxylic acid cycle, leaving at most 20% of total entry to go directly to glucose. This final number of 20% is not inconsistent with the assessment that most of the net formation of oxaloacetate formed from propionate is used for glucose formation, provided that none of these cycles has a large nonglucogenic efflux. In order for this cycle to be active with no net loss of glucogenic carbon, pyruvate carboxylase and pyruvate kinase must have activity exceeding net gluconeogenic rates and pyruvate dehydrogenase (PDH) activity must be low. Analysis of sheep liver reveals that pyruvate kinase activity is roughly ten times that of phosphoenolpyruvate carboxykinase and pyruvate carboxylase, although the activity of pyruvate kinase drops to thrice pyruvate carboxylase in late pregnancy (27). In cattle the activity of phosphoenolpyruvate carboxykinase (combined mitochondrial and cytosolic) and pyruvate carboxylase are approximately equal (28). Although total PDH activity in sheep liver is lower than in rats, the proportion in active form was higher, resulting in similar amounts of active PDH in sheep and rat liver (29). An active pyruvate kinase and PDH in ruminant liver, which oxidizes essentially no glucose, is puzzling but may account for what appears to be extensive oxidation of lactate discussed below. Experiments with bovine liver in vitro also support extensive cycling of propionate carbon through oxaloacetate, phosphoenolpyruvate and pyruvate, because 20% of labeled propionate is recovered in lactate (30) and lactate carbon is recovered in glucose (7).

The energetic cost of this futile cycling in domestic ruminants can be estimated. First, we assume all of the net glucose output by the liver arises from oxaloacetate and set the ratio of oxaloacetate going through this cycle versus going to glucose as 0.5/0.2 = 2.5; this ratio being derived from sheep near maintenance as discussed above. We then use values of hepatic oxygen uptake of 3061 mmol/h (9) and glucose production of 713 mmol glucose/h measured si-

multaneously in lactating cows (20). This implies 1426 mmol of oxaloacetate/h flowing to glucose and 3565 mmol oxaloacetate/h passing through the futile cycle. At a cost of 1 ATP per cycle and 6 ATP per oxygen consumed, this cycle alone accounts for 19.4% of hepatic oxygen consumption. If liver accounts for 22% of whole-body energy metabolism in lactating cows (31), then elimination of this cycle would decrease whole-animal energy utilization by 4.3%. Most advances in gross animal efficiency have been based on increases in production, which dilute maintenance costs over a larger return. In an animal using 33% of its metabolizable intake energy for maintenance and the rest for productive purposes, production would have to increase by ~10% to give the same improvement in gross efficiency as a 4.3% decrease in oxygen consumption. This cycle can also affect the energetic efficiency of nonproductive or slowly growing animals (dairy replacements, dry cows and wintering brood animals), which form a large portion of the herd's maintenance costs and whose individual efficiency cannot be altered by increased production but can be altered by lowering maintenance costs. Glucose output and oxygen consumption data from steers (Reynolds, USDA Ruminant Nutrition Laboratory, Beltsville MD personal communication) gives a value of 17%, rather than 22%, of hepatic oxygen consumption attributable to this cycle. Therefore this cycle represents a possible target for improving utilization of feed energy in a wide range of domestic ruminants by a significant, albeit finite, amount.

The presence of this futile cycling pathway, and the assumption of complete conversion of propionate carbon to glucose in vivo, imply that treatments that increase flow of phosphoenolpyruvate towards glucose may not increase the real conversion of propionate carbon to glucose in vivo, but may increase the energetic efficiency of this process instead. Therefore, glucagon and growth hormone, both of which increase propionate conversion to glucose in vitro (4, 7, 32), may improve energetic efficiency of gluconeogenesis in vivo. Butyrate inhibits propionate conversion to glucose in vitro (15), and, if it acts at a point beyond phosphoenolpyruvate, it may decrease the energetic efficiency of gluconeogenesis.

Although the in vivo quantitation of oxaloacetate cycling through pyruvate kinase on its way to glucose is consistent with measured enzyme activities and in vitro liver metabolic fluxes, several important questions remain. Ruminant liver may contain active PDH yet conserve propionate carbon only if there is either chronic inhibition of PDH in vivo that is not dependent on phosphorylation (33) or some compartmental separation of the oxidative and gluconeogenic pathways. Localization of PDH pericavally in the liver lobule would permit constant gluconeogenesis in the periportal cells, with no potential for loss of gluconeogenic carbon to oxidation in the citric acid cycle. If such localization did exist, substances that interfere with propionate removal by the periportal cells of the lobule could increase the oxidative loss of propionate carbon by shifting its metabolism pericavally. Another problem that must be addressed is that, if propionate carbon passes through pyruvate, differences in rate of lactate and propionate metabolism must imply a limitation in conversion of extracellular lactate into the pyruvate pool carboxylated by pyruvate carboxylase.

Lactate conversion to glucose compared with other fates has not been as carefully corrected for the errors in label transfer as the data discussed for propionate. The magnitude of correction required should be similar, depending on the label location within lactate, because oxaloacetate is a common intermediate for propionate and lactate. The fraction of lactate turnover being converted to glucose has been calculated to be 30–34% in pregnant sheep and lactating cows, but only 16% in lactating sheep and 27% in pregnant cows (34). Even the higher numbers are less than the uncorrected 63% calculated for propionate (24) and therefore indicate that a significant portion of lactate is in fact not used for glucose. If only hepatic metabolism of lactate is considered, the data indicates that less than one-fourth of hepatic lactate uptake is used for glucose synthesis (21). Because lactate is presented to the pericaval cells of the hepatic lobule in much greater concentration than is propionate and lactate appears to be more extensively oxidized, it is tempting to speculate that PDH may be located pericavally, thereby sparing propionate completely from true oxidation, but not lactate. Although gluconeogenesis is localized periportally in other species, neither this nor any other aspect of lobular zonal heterogeneity has been tested in ruminant liver. Many of the functions located pericavally in those species that have been studied, such as glycogenolysis and lipogenesis, are not present in ruminant liver at all. The possible segregation of metabolic activities within the liver lobule of ruminants is clearly an important facet of regulation requiring research attention.

LITERATURE CITED

1. Elliot, J. M. (1980) Propionate metabolism and vitamin B_{12}. In: Digestive Physiology and Metabolism in Ruminants (Ruckebush, Y. & Thivend, P., eds.), pp. 485–503, AVI Publishing, Westport, CT.
2. Giesecke, D. & Stangassinger, M. (1980) Lactic acid metabolism. In: Digestive Physiology and Metabolism in Ruminants (Ruckebush, Y. & Thivend, P., eds.), pp. 523–539, AVI Publishing, Westport, CT.
3. Stangassinger, M. & Giesecke, D. (1986) Splanchnic metabolism of glucose and related energy substrates. In: Control of Digestion and Metabolism in Ruminants (Milligan, L. P., Grovum, W. L. & Dobson, A., eds.), pp. 347–366, Prentice-Hall, Englewood Cliffs, NJ.
4. Donkin, S. S., Armentano, L. E. & Bertics, S. J. (1990) Short-term effects of glucagon or insulin, and long-term effects of in-

sulin on bovine hepatic gluconeogenesis from propionate in vitro. J. Dairy Sci. 73(suppl. 1): 136(abs.).
5. Faulkner, A. & Pollock, H. T. (1990) Effects of glucagon and a- and b-agonists on glycogenolysis and gluconeogenesis in isolated ovine hepatocytes. Biochim. Biophys. Acta 1052: 229–234.
6. Zammit, V. A. (1990) Triacylglycerol synthesis in cultured sheep liver cells. Hannah Research Institute Report, Ayr, Scotland.
7. Donkin, S. S., Armentano, L. E. & Bertics, S. J. (1991) Gluconeogenesis from propionate, lactate and glycerol in bovine hepatocytes: Insulin and glucagon effects. FASEB J. 5: A757(abs.).
8. Bergman, E. N. & Wolff, J. E. (1971) Metabolism of volatile fatty acids by liver and portal-drained viscera in sheet. Am. J. Physiol. 221: 586–592.
9. Reynolds, C. K., Huntington, G. B., Tyrrell, H. F. & Reynolds, P. J. (1988) Net metabolism of volatile fatty acids, d-b-Hydroxybutyrate, nonesterified fatty acids, and blood gasses by portal-drained viscera and liver of lactating holstein cows. J. Dairy Sci. 71: 2395–2405.
10. Demigné, C., Yacoub, C., Rémésy, C. & Fafournoux, P. (1986) Propionate and butyrate metabolism in rat or sheep hepatocytes. Biochim. Biophys. Acta 875: 535–542.
11. Ricks, C. A. & Cook, R. M. (1981) Regulation of volatile fatty acid uptake by mitochondrial acyl CoA synthetases of bovine liver. J. Dairy Sci. 64: 2324–2335.
12. Knowles, S. E., Jarrett, I. G., Filsell, O. H. & Ballard, F. J. (1974) Production and utilization of acetate in mammals. Biochem. J. 142: 401–405.
13. Pethick, D. W., Lindsay, D. B., Barker, P. J. & Northrop, A. J. (1981) Acetate supply and utilization by the tissues of sheep in vivo. Br. J. Nutr. 46: 97–110.
14. Lomax, M. A. & Baird, G. D. (1983) Blood flow and nutrient exchange across the liver and gut of the dairy cow: Effects of lactation and fasting. Br. J. Nutr. 49: 481–496.
15. Aiello, R. J., Armentano, L. E., Bertics, S. J. & Murphy, A. T. (1989) Volatile fatty acid uptake and propionate metabolism in ruminant hepatocytes. J. Dairy Sci. 72: 942–949.
16. Looney, M. C., Baldwin, R. L. & Calvert, C. C. (1987) Gluconeogenesis in isolated lamb hepatocytes. J. Anim. Sci. 64: 283–294.
17. Aiello, R. J. & Armentano, L. E. (1987) Effects of volatile fatty acids on propionate metabolism and gluconeogenesis in caprine hepatocytes. J. Dairy Sci. 70: 2504–2510.
18. Garton, G. A., Hovell, F. D. DeB. & Duncan, W. R. H. (1972) Influence of dietary volatile fatty acids on the fatty-acid composition of lamb triglycerides, with special reference to the effect of propionate on the presence of branched-chain components. Br. J. Nutr. 28: 409–416.
19. Armentano, L. E., Grummer, R. R., Bertics, S. J., Skaar, T. C. & Donkin, S. S. (1991) Effects of energy balance on hepatic capacity for oleate and propionate metabolism and triglyceride secretion. J. Dairy Sci. 74: 132–139.
20. Reynolds, C. K., Huntington, G. B., Tyrrell, H. F. & Reynolds, P. J. (1988) Net portal-drained visceral and hepatic metabolism of glucose, L-lactate, and nitrogenous compounds in lactating holstein cows. J. Dairy Sci. 71: 1803–1812.
21. Van Der Walt, J. G., Baird, G. D. & Bergman, E. N. (1983) Tissue glucose and lactate metabolism and interconversions in pregnant and lactating sheep. Br. J. Nutr. 50: 267–280.
22. Aiello, R. J. & Armentano, L. E. (1987) Gluconeogenesis in goat hepatocytes is affected by calcium, ammonia and other key metabolites but not primarily through cytosolic redox state. Comp. Biochem. Physiol. 88B: 193–201.
23. Gross, K. L., Harmon, D. L., Minton, J. E. & Avery, T. B. (1990) Effects of isoenergetic infusions of propionate and glucose on portal-drained visceral nutrient flux and concentrations of hormones in lambs maintained by total intragastric infusion. J. Anim. Sci. 68: 2566–2574.
24. Steinhour, W. D. & Bauman, D. E. (1988) Propionate metabolism: A new interpretation. In: Aspects of Digestive Physiology in Ruminants (Dobson, A. & Dobson, M. J., eds.), pp. 238–256, Comstock, Ithaca, NY.
25. Veenhuizen, J. J., Russell, R. W. & Young, J. W. (1988) Kinetics of metabolism of glucose, propionate and CO_2 in steers as affected by injecting phlorizin and feeding propionate. J. Nutr. 118: 1366–1375.
26. Armentano, L. E. & Young, J. W. (1983) Production and metabolism of volatile fatty acids, glucose and CO_2 in steers and the effects of monensin on volatile fatty acid kinetics. J. Nutr. 113: 1265–1277.
27. Smith, R. W. & Walsh, A. (1982) Effects of pregnancy and lactation on the activities in sheep liver of some enzymes of glucose metabolism. J. Agric. Sci. (Camb.) 98: 563–565.
28. Baird, G. D. & Young, J. L. (1975) The response of key gluconeogenic enzymes in bovine liver to various dietary and hormonal regimes. J. Agric. Sci. (Camb.) 84: 227–230.
29. Robertson, J. P., Faulkner, A. & Vernon, R. G. (1980) Pyruvate dehydrogenase and the regulation of glucose metabolism in ruminant tissues. FEBS Lett. 120: 192–194.
30. Knapp, J. R., Freetly, H. C., Reis, B. L., Calvert, C. C. & Baldwin, R. L. (1989) Effects of sometribove on patterns of liver metabolism in dairy cattle with varying levels of production. J. Dairy Sci. 67(suppl. 1): 326(abs.).
31. Smith, N. E. & Baldwin, R. L. (1974) Effects of breed, pregnancy, and lactation on weight or organs and tissues in dairy cattle. J. Dairy Sci. 57: 1055–1060.
32. Pocius, P. A. & Herbein, J. H. (1986) Effects of in vivo administration of growth hormone on milk production and in vitro hepatic metabolism in dairy cattle. J. Dairy Sci. 69: 713–720.
33. Stansbie, D., Denton, R. M., Bridges, B. J., Pask, H. T. & Randle, P. J. Regulation of pyruvate dehydrogenase and pyruvate dehydrogenase phosphate phosphatase in rat epididymal pads. Biochem. J. 154: 225–236.
34. Baird, G. D., Van Der Walt, J. G. & Bergman, E. N. (1983) Whole-body metabolism of glucose and lactate in productive sheep and cows. Br. J. Nutr. 50: 249–265.