Net Hepatic and Splanchnic Metabolism of Lactate, Pyruvate and Propionate in Dairy Cows *in vivo* in Relation to Lactation and Nutrient Supply

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1. Circulating concentrations of glucose, propionate, lactate and pyruvate, and net exchange of these compounds across the liver and gut, were measured in lactating and non-lactating dairy cows (*a*) in the normal fed state, (*b*) before, during and after intravenous infusion of an aqueous solution of glucose, propionate or lactate (lactating cows only) in fed animals, and (*c*) before and during 6 days of food deprivation. 2. In the normal fed state, gut output of propionate, hepatic output of glucose and hepatic uptake of lactate were all higher in the lactating group. There was a net uptake of pyruvate across the liver in the lactating cows and a net output in the non-lactating cows. In the lactating cows there was a net uptake of lactate and pyruvate by the splanchnic bed (i.e. gut and liver combined). 3. In the lactating cows, the glucose and propionate infusions had the following effects: decrease in net hepatic uptake of lactate; a switch in pyruvate exchange across the liver from uptake to output; suppression of uptake of lactate and pyruvate by the splanchnic bed; increase in the magnitude of the liver (propionate uptake)/(glucose output) ratio. Lactate infusion did not affect hepatic propionate uptake. 4. Food deprivation increased hepatic extraction of lactate and pyruvate and decreased the liver (propionate uptake)/(glucose output) ratio in both groups. 5. It is concluded that mechanisms exist to ensure an inverse relationship between the availability to the cow of glucose or propionate and utilization by the splanchnic bed of endogenously derived lactate and pyruvate.

The major portion of the glucose available to fed or food-deprived ruminants is provided by hepatic gluconeogenesis (see, e.g., Bergman, 1973), and the rate of this process can therefore be taken to be approximately equivalent to glucose-entry rate, i.e. the rate of entry of glucose into the systemic circulation. Hepatic gluconeogenesis can take place from a variety of precursors which are either of exogenous or endogenous origin. Three of the compounds that are taken up by the liver and can act as precursors for glucose formation are propionate, lactate and pyruvate (Bergman, 1973; Thompson *et al.*, 1975; Clark *et al.*, 1976). Propionate is clearly an exogenous precursor, since the only propionate available to the animal is that which is absorbed from the gut. Lactate and pyruvate, on the other hand, are precursors that can be of exogenous or endogenous origin. Thus earlier studies have shown that there is a consistent output of lactate from the gut of the fed dairy cow (Baird *et al.*, 1975), and this lactate could be of exogenous or endogenous origin, depending on whether the lactate is derived from a product of

digestion or from arterial glucose by glycolysis. However, the liver can also take up lactate and pyruvate, which are derived from outside the splanchnic bed, i.e. from outside the gut and liver acting together as a functionally integrated organ. The lactate and pyruvate in this case must clearly be of endogenous origin.

The rate at which hepatic gluconeogenesis takes place in the ruminant depends on reproductive and nutritional status. The influence of reproductive status is demonstrated by the fact that in dairy cows hepatic gluconeogenesis, as measured either by glucose-entry rate or hepatic glucose output, is higher in lactating animals than in non-lactating animals (e.g. Leng, 1970; Paterson & Linzell, 1974; Lomax *et al.*, 1979). The influence of nutritional status is evident from the observation that hepatic glucose output decreases in both lactating and non-lactating dairy cows during food deprivation (e.g. Lindsay, 1970; this paper). The influence of nutrition is also apparent in the effects of the administration of glucose or propionate to fed animals. Thus, in wether sheep, intrajugular infusion of glucose can elicit a decrease in endogenous glucose entry (Judson & Leng, 1973*a*), whereas infusion of propionate via a mesenteric

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vein leads to an increase in this parameter (Judson & Leng, 1973b). Glucose infusion has also been shown to decrease endogenous glucose entry and hepatic glucose output in lactating cows (Thompson *et al.*, 1975; Lomax *et al.*, 1979), whereas short-term propionate infusion has been shown to increase hepatic glucose output in non-lactating cows (Lomax *et al.*, 1979).

Physiological and nutritional status will clearly also determine the total demand for gluconeogenic precursors, and the extent of the demand for endogenous precursors. In this context it was of interest to determine in the dairy cow *in vivo* how the hepatic uptake of propionate, lactate and pyruvate varied in relation to lactation and to nutritional status. The three compounds are all metabolized to the same intermediate, i.e. oxaloacetate, before entering the final gluconeogenic pathway. In the case of propionate, transformation to oxaloacetate occurs via propionyl-CoA, methylmalonyl-CoA, succinyl-CoA and the tricarboxylic acid cycle. Lactate, by contrast, is transformed directly via pyruvate to oxaloacetate. Competition between propionate, on the one hand, and lactate and pyruvate, on the other, for uptake by the liver could therefore arise, and has in fact already been demonstrated in other species *in vitro* (Smith & Osborne-White, 1971; Chan & Freedland, 1972).

A preliminary report of some of these findings has been published (Baird *et al.*, 1978).

Experimental

Materials

p-Aminohippuric acid, used for the determination of blood flow rate, was obtained from Sigma (London) Chemical Co., Kingston upon Thames, Surrey, U.K., as was L(+)-lactic acid [30% (w/v) aqueous soln.; high purity]. Propionic acid (99% pure) was obtained from Koch-Light Laboratories, Colnbrook, Bucks., U.K., and glucose (of AnalaR grade) was obtained from BDH Chemicals, Poole, Dorset, U.K. Double-distilled water, the second distillation being from glass, was used throughout.

Nine healthy mature non-pregnant Friesian $\times$ Ayrshire dairy cows were used, each of which had calved on at least two occasions. At the time of the experiment, five of the cows were lactating and four non-lactating. All the animals had been catheterized surgically for the measurement of portal and hepatic metabolite production rates by a combination of the methods of Symonds & Baird (1973) and Baird *et al.* (1975). The catheterization involved placing catheters in a mesenteric vein, the portal vein at the porta hepatis, a hepatic vein and a carotid artery in each cow. The animals were allowed to recover from surgery for at least 3 weeks, and to return to normal feeding, before being used in the experiments.

For the glucose-infusion studies, a catheter was also placed in a jugular vein at least 48 h before an experiment. When feeding normally, the non-lactating cows were receiving about 5 kg of medium-quality hay and 3 kg of dairy concentrate per day, and the lactating cows were receiving in addition 0.3 kg of dairy concentrate/kg of milk produced. The daily feed was given in two equal portions, the first at about 07:30 h and the second at about 14:30 h. At the time the glucose-infusion experiments were being carried out the lactating cows were in their 2nd or 3rd month of lactation and were giving an average milk yield of about 17.3 kg/day. The average daily intake of metabolizable energy by each animal was calculated to be about 122 MJ for the lactating cows and 66 MJ for the non-lactating cows. These intakes provided about 93 and 122% of requirement plus safety margin for the two groups of cows respectively, according to Technical Bulletin 33 (1975).

Methods

Experimental procedure. Circulating concentrations and net hepatic and portal production rates of lactate, pyruvate, glucose and propionate were measured in both lactating and non-lactating cows in the following situations: (1) normal fed state; (2) before, during and after intravenous infusion of (a) glucose and (b) propionate; (3) before and during food deprivation. Additionally, some of the above parameters were also measured in lactating cows (4) before, during and after intravenous infusion of lactate.

Situations (1), (2a) and (2b). The procedures adopted were those of Lomax *et al.* (1979), the data given in the present paper being obtained from the same experiments as those described by these authors. In situation (2a) 50% (w/v) glucose was infused at 1.5 ml (4.2 mmol)/min for 48 h via the jugular-vein catheter, and in situation (2b) 5.0 M-sodium propionate was infused at 2.0 ml (10 mmol)/min for 3 h via the mesenteric-vein catheter. In both the glucose- and propionate-infusion experiments, three each of the lactating and non-lactating cows were given the appropriate experimental infusion and two each the corresponding control infusion. This latter consisted of water at 1.5 ml/min and 5.0 M-NaCl at 2 ml/min in the glucose and propionate experiments respectively.

Situation (3). Two lactating and two non-lactating cows were used for investigation of the effect of food deprivation for 6 days (144 h). The food-deprivation experiment was the same as that reported by Baird *et al.* (1979), and the procedure was as described by them.

Situation (4). Infusion of lactate was carried out in a similar manner to the infusion of propionate

[situation (2b)], except that instead of the propionate solution, a neutral aqueous solution of 2.5M-sodium lactate was infused at a constant rate of 1.4ml (3.5mmol)/min via the mesenteric-vein catheter. Two cows were infused with lactate and two control cows were infused with 2.5M-NaCl under otherwise identical conditions.

Selection of infusion parameters in situations (2a), (2b) and (4). In general, the rates chosen were intended to elicit a doubling of previously observed rates in untreated animals (cf. Baird *et al.*, 1975). Thus the rate of glucose infusion was approximately the mean of hepatic glucose output previously observed in lactating and non-lactating cows, and those of the propionate and lactate infusions were the same as the gut output and hepatic uptake respectively seen in lactating cows (Baird *et al.*, 1975). Glucose was infused for 48h in order to make the present work comparable with earlier studies (Treacher *et al.*, 1976). Propionate and lactate were infused for only 3h in order to avoid any interference with appetite or pathological effects arising from the infusion of Na⁺. In the event, none of the experimental infusions had any effect on appetite, and the control infusions had no statistical effect on either appetite or measured parameters.

Blood. Blood samples were removed by syringe via the catheters implanted in the carotid artery, the portal vein and the hepatic vein, and collected at 0°C into heparin for the determination of *p*-amino-hippurate concentration and haematocrit, and into 6% (w/v) HClO₄ for the determination of lactate, pyruvate, glucose and propionate. A portion of the heparinized blood was centrifuged and the plasma removed and stored frozen before assay. The concentration of *p*-aminohippurate in the plasma was subsequently determined by the method of Harvey & Brothers (1962). Haematocrit was determined in the remainder of the blood sample by using a Coulter Counter (Coulter Diagnostics, Hialeah, FL, U.S.A.). The HClO₄ extracts of blood were neutralized with 20% (w/v) KOH as described previously (Baird & Heitzman, 1970). Pyruvate was assayed in the fresh neutralized extract on the day of collection. For the other metabolites, the extracts were stored frozen until assayed. The concentrations of lactate and pyruvate in the extracts were determined by using lactate dehydrogenase (EC 1.1.1.27) by the methods of Gutmann & Wahlefeld (1974) and Czok & Lamprecht (1974) respectively, and that of glucose was determined by the method of Bergmeyer *et al.* (1974). The concentration of propionate in the extracts was determined, after reacidification, by g.l.c. in a series 204 gas chromatograph (Pye-Unicam, Cambridge, U.K.). The column was that used by Baird *et al.* (1975).

Calculations. Portal and hepatic plasma flow rates were calculated from the plasma *p*-aminohippurate

concentrations. Whole-blood flow rates were then obtained by multiplying plasma flow rates by 1/(1-haematocrit) (see Katz & Bergman, 1969). The method gives values for blood flow through the portal vein and the liver. Flow through the hepatic artery is then determined by difference. Flow rates are higher during lactation. For example, the mean values for portal and liver whole-blood flow before infusion in the glucose experiments were respectively 23.5 and 28.3 litres/min for the lactating cows and 17.0 and 21.7 litres/min for the non-lactating cows. Net portal and hepatic production rates of the various metabolites were obtained from the appropriate whole-blood flow rates and from the appropriate metabolite concentrations in whole blood (Katz & Bergman, 1969). A positive production rate indicates net output, and a negative production rate indicates net uptake. Rate of arrival of a metabolite at the liver was calculated by using the formula

$$Ar = F_P C_P + F_{HA} C_A$$

where *Ar* is the rate of arrival in mmol/min, *F_P* and *F_{HA}* are the whole-blood flow rates in litres/min in the portal vein and hepatic artery respectively, and *C_P* and *C_A* are the whole-blood concentrations of the metabolite in mmol/l in the portal vein and carotid artery respectively. Percentage hepatic extraction of a metabolite was given by:

$$E = \frac{(F_P C_P + F_{HA} C_A) - (F_{HV} C_{HV})}{F_P C_P + F_{HA} C_A} \times 100$$

where *E* is percentage hepatic extraction, and *F_{HV}* and *C_{HV}* are the whole-blood flow rate in litres/min and the whole-blood concentration of the metabolite in mmol/l respectively in the hepatic veins. A positive extraction indicates net uptake and a negative extraction indicates net output.

Control of pyrogenicity of infused solutions. This was carried out as described by Lomax *et al.* (1979).

Statistics. Standard errors calculated from the mean values for individual cows were used to test the significances of differences in parameters of glucose, propionate, lactate and pyruvate metabolism between lactating and non-lactating cows (Tables 1 and 2). In the glucose- and propionate-infusion experiments a 2 × 2 factorial split-plot-in-time analysis of variance was performed on the data, with the main-plot effects being (a) glucose or propionate infusion versus appropriate control infusion, and (b) lactating cows versus non-lactating cows, and the sub-plot effect being the time of sampling. In the lactate-infusion experiment the treatment was similar, except that the only main-plot effect was lactate infusion versus control infusion. In the food-deprivation experiment a split-plot analysis of variance was also performed, but with lactating cows versus non-lactating cows as the only main-plot

Table 1. *Parameters of glucose, insulin and propionate metabolism in lactating and non-lactating dairy cows in the normal fed state*

The parameters are the means of values obtained for five lactating cows and four non-lactating cows in the case of glucose and insulin, and four lactating cows and three non-lactating cows in the case of propionate. The values obtained for individual cows were in turn means of several observations. ** $P < 0.01$ and *** $P < 0.001$ as compared with corresponding values for lactating cows. The insulin values are taken from Lomax *et al.* (1979).

Metabolite	Parameter	Units	Lactating	Non-lactating	Standard error of difference between means
Glucose	Arterial concentration	mm	2.73	3.27***	0.10
	Gut production	mmol/min	-0.28	+0.10	0.49
	Liver production		+6.54	+4.00**	0.69
Insulin	Arterial concentration	m-i.u./litre	14.5	32.3***	2.7
Propionate	Portal concentration	mm	0.36	0.35	0.02
	Gut production	mmol/min	+7.47	+4.88**	0.48
	Liver production		-6.91	-4.53**	0.51

Table 2. *Parameters of lactate and pyruvate metabolism in lactating and non-lactating cows in the normal fed state*
 Rate of uptake of lactate by the liver in the lactating cows is significantly ($P < 0.05$) higher than the corresponding rate of output of lactate by the gut. The parameters of lactate metabolism are the means of values obtained for five lactating cows and four non-lactating cows. Those for pyruvate metabolism are the means of values obtained for four lactating cows and three non-lactating cows. The values obtained for individual cows are in turn means of several observations. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ as compared with corresponding values for lactating cows.

Metabolite	Parameter	Units	Lactating	Non-lactating	Standard error of difference between means
Lactate	Arterial concentration	mm	0.297	0.420**	0.028
	Portal-vein concentration		0.378	0.515***	0.017
	Gut production	mmol/min	+1.76	+1.53	0.27
	Liver production		-2.99	-0.85**	0.55
	Arrival at liver		8.29	8.52	0.75
	Hepatic extraction	%	29.6	8.9**	4.7
Pyruvate	Arterial concentration	mm	0.018	0.030*	0.004
	Gut production	mmol/min	+0.07	-0.01	0.04
	Liver production		-0.23	+0.16**	0.06

effect, and with day of sampling as the sub-plot effect.

Results and Discussion

Metabolism in normal fed cows

Table 1 shows that, in accordance with previous observations made on lactating and non-lactating ruminants (e.g. Bergman & Hogue, 1967; Leng, 1970), the arterial concentration of glucose was lower in the lactating cows than in the non-lactating cows, while the hepatic output of glucose (essentially equivalent to glucose-entry rate) was higher. As previously reported (Lomax *et al.*, 1979), the arterial insulin concentration was also lower in the lactating cows. Finally, Table 1 shows that the rates of gut output and hepatic uptake of propionate were higher in the lactating animals. This would be expected, since the greater feed intake of the lactating cows (see the Experimental section) would ensure that gut output of propionate was greater in this group, whereas in both groups of cows the liver extracted between 80 and 85 % of the propionate presented to it.

Table 2 shows that the arterial and portal-vein concentrations of lactate were lower in the lactating cows than in the non-lactating cows, whereas hepatic uptake and extraction of lactate were higher. Despite these differences, the rate of output of lactate from the gut and the rate of arrival of lactate at the liver were very similar in the two groups of cows, since portal-venous and hepatic blood-flow rates were both higher in the lactating cows (see the Experimental section). The parameters of pyruvate metabolism varied between the lactating and non-lactating cows in an analogous manner to those of lactate metabolism (Table 2). The lactate that was absorbed from the gut could have arisen in three possible ways, i.e. (i) conversion of an absorbed metabolite during passage across the gut wall, (ii) absorption of lactate from the gut lumen or (iii) glycolysis of arterial glucose. The greater hepatic uptake of lactate in the lactating cows could be due to a greater activity of either liver pyruvate carboxylase (EC 6.4.1.1) or pyruvate dehydrogenase (EC 1.2.4.1), or even to the fact that the liver may be larger during lactation. Ballard *et al.* (1968) observed that the hepatic activity of

Table 3. *Effect of infusion of either glucose or propionate on net hepatic production of glucose in lactating and non-lactating cows*
 Net hepatic production is expressed as mmol/min. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, as compared with pre-infusion value. Propionate-infusion data taken from Lomax *et al.* (1979).

Cow status	Treatment	Pre-infusion	Infusion			Post-infusion	Standard error of difference between means
			4.5h	24h	48h		
(a) Glucose infusion							
Lactating	Glucose-infused	6.52	1.14***	2.62**	4.24	7.77	1.10
	Control	5.44	8.71*	5.46	6.61	5.46	1.35
Non-lactating	Glucose-infused	3.58	3.18	1.36	4.83	4.23	1.10
	Control	3.65	5.66	2.51	3.55	2.48	1.35
			0.5h	1.5h	3.0h		
(b) Propionate infusion							
Lactating	Propionate-infused	6.74	5.12	6.99	9.22	7.72	1.21
	Control	6.11	6.18	5.81	7.00	7.60	1.49
Non-lactating	Propionate-infused	3.72	8.10**	8.89**	7.26*	4.52	1.21
	Control	2.17	2.09	3.61	2.36	5.47*	1.49

pyruvate carboxylase *in vitro* was greater in lactating cows than in non-lactating cows.

It is evident from Table 2 that there was a difference between the lactating and non-lactating cows in the direction of net exchange of lactate and pyruvate across the splanchnic bed. In the lactating cows there was a net uptake of these compounds, whereas in the non-lactating cows there was a net output. This indicates that there must have been a net flow of lactate and pyruvate from peripheral tissues to the splanchnic bed in the lactating cows. This endogenously derived lactate and pyruvate must presumably have arisen by glycolysis from glucose or glycogen in peripheral tissue, and must therefore represent Cori-cycle activity, which was not occurring in the non-lactating cows. The recycling of lactate and pyruvate in the lactating cows presumably represents a means for conserving glucose carbon in these animals. Conservation of glucose will be important in view of the high glucose demand of the lactating mammary gland, which can utilize some 70% of total glucose entry (Bickerstaffe *et al.*, 1974). The glucose taken up by the gland is used mainly to provide both the glucose and galactose moieties of lactose, and for oxidative purposes (Davis & Bauman, 1974). As in non-ruminants (Robinson & Williamson, 1977), there is a net uptake of lactate by the lactating mammary gland of fed ruminants (Linzell, 1974), and it is clear that in the lactating cows in the present study this lactate must have been provided from other peripheral tissues, and not from the splanchnic bed.

The release of lactate and pyruvate from peripheral tissues in the lactating cows could be due to the fact that the low circulating concentration of insulin (Table 1) gives rise to a partial inhibition of pyruvate dehydrogenase in these tissues, either directly, as in the case of adipose tissue (Coore *et al.*, 1971), or indirectly via a rise in the blood concentration of

non-esterified fatty acids, and hence an increase in the peripheral tissue [acetyl-CoA]/[CoA] ratio (see, e.g., Newsholme & Start, 1973). As a result of the recycling, the proportion of assimilated glucose carbon that entered the tricarboxylic acid cycle would decrease during lactation in peripheral tissues other than the mammary gland. In this connection Bartley & Black (1966) and Bennink *et al.* (1972) have reported that the percentage contribution of glucose to CO₂ output is decreased in cows during lactation.

Effects of infusion of glucose, propionate or lactate

As Table 3 shows, the glucose infusion elicited a decrease in hepatic glucose output in the lactating cows, but not in the non-lactating cows. By contrast, the propionate infusion had little or no effect on hepatic glucose output in the lactating animals, but produced a substantial and sustained increase in this output in the non-lactating animals. It is evident therefore, as has already been pointed out (Lomax *et al.*, 1979), that the two infusions increased total glucose-entry rate (i.e. rate of hepatic glucose output + rate of glucose infusion, when this occurred) in the non-lactating cows, but not in the lactating cows.

Table 4 records the effects of the two infusions on the arterial concentrations of glucose and insulin in the two groups of cows. Generally speaking, the infusions caused only relatively moderate increases in glucose concentration in both groups, and in this respect therefore the response of the two groups of cows was similar. On the other hand, there were marked differences between the two groups in the effect of the infusions on insulin concentration. While both infusions elicited large increases in insulin concentration in the non-lactating cows, the glucose infusion caused only a moderate and transient increase in insulin concentration in the lactating

Table 4. *Effect of infusion of either glucose or propionate on arterial concentrations of glucose and insulin in lactating and non-lactating cows*

Concentration of glucose is expressed as mM in whole blood and of insulin as m-i.u./litre in plasma. The data are taken from Lomax *et al.* (1979). Control values have been omitted for clarity, but were used in the statistical analysis. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ as compared with the pre-infusion value.

Metabolite	Cow status	Pre-infusion	Infusion			Post-infusion	Standard error of difference between means
			4.5h	24h	48h		
(a) Glucose infusion							
Glucose	Lactating	2.86	4.26***	3.42*	3.27	2.72	0.24
	Non-lactating	3.25	3.91*	3.81*	3.87*	3.31	0.24
Insulin	Lactating	16.5	36.2	17.7	18.9	15.1	18.6
	Non-lactating	37.0	130.1***	145.6***	145.7***	47.8	18.6
(b) Propionate infusion							
Glucose	Lactating	2.62	2.88*	2.99*	2.98*	3.03*	0.13
	Non-lactating	3.34	3.44	3.55	3.32	3.44	0.13
Insulin	Lactating	12.4	15.2	14.6	17.1	13.3	17.2
	Non-lactating	45.7	110.1**	94.4*	47.5	39.7	17.2

Table 5. *Effect of infusion of glucose or propionate on net hepatic production of lactate in lactating and non-lactating cows*
Hepatic production rates are expressed as mmol/min. * $P < 0.05$ and ** $P < 0.01$ as compared with pre-infusion value.

Cow status	Treatment	Pre-infusion	Infusion			Post-infusion	Standard error of difference between means
			4.5 h	24 h	48 h		
(a) Glucose infusion							
Lactating	Glucose-infused	-3.34	-3.88	-1.56*	-0.99**	-2.47	0.72
	Control	-2.53	-3.31	-1.74	-2.71	-2.42	0.88
Non-lactating	Glucose-infused	-0.67	-0.42	-0.37	-0.46	-0.67	0.72
	Control	-0.48	-1.54	-0.67	-0.32	-0.67	0.88
			0.5 h	1.5 h	3.0 h		
(b) Propionate infusion							
Lactating	Propionate-infused	-3.07	-1.78*	-1.70*	-1.68*	-2.70	0.60
	Control	-2.78	-1.89	-2.79	-1.80	-1.58	0.73
Non-lactating	Propionate-infused	-0.38	+0.29	+0.39	+0.46	+0.01	0.60
	Control	-0.40	-0.48	-0.22	-0.35	-0.49	0.73

cows, and the propionate infusion had no effect at all in this group. The significance of these observations has been discussed in detail elsewhere (Lomax *et al.*, 1979).

As Table 5(a) shows, the glucose infusion elicited a significant decrease in net hepatic uptake of lactate in the lactating cows at 24 and 48h of infusion, but not at 4.5h. By contrast, the infusion had no apparent effect on hepatic lactate uptake in the non-lactating cows. The failure of glucose to decrease hepatic lactate uptake in the lactating cows at 4.5h of infusion may have been related to the fact that the cows had received their afternoon feed shortly before sampling, since hepatic lactate uptake and extraction were greatest at this time in the control cows (Tables 5 and 7). The propionate infusion caused a decrease in net hepatic uptake of lactate in both groups of cows (Table 5b). This decrease was significant at all times for the lactating cows and at 1.5 and 3h for the

lactating and non-lactating cows taken together ($P < 0.05$).

Although not tabulated, it was further observed that neither the glucose nor propionate infusions had any effect on gut lactate output. In both experiments this output amounted on average to 1.9 and 1.4mmol/min in the lactating and non-lactating cows respectively. Both the glucose and propionate infusions decreased hepatic lactate uptake in the lactating cows to values less than 1.9mmol/min (Table 5). It is clear therefore that during these infusions the net uptake of lactate by the splanchnic bed that occurs in untreated lactating cows (Table 2) must have ceased.

As Table 6(a) shows, there was little change in arterial lactate concentration in the lactating cows during the glucose infusion. The increase in the non-lactating cows at 4.5h may have been related to feeding, although no corresponding increase was

Table 6. *Effect of infusion of glucose or propionate on the arterial concentration of lactate in lactating and non-lactating cows*
Concentration is expressed as mM. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ as compared with pre-infusion value.

Cow status	Treatment	Pre-infusion	Infusion			Post-infusion	Standard error of difference between means
			4.5 h	24 h	48 h		
(a) Glucose infusion							
Lactating	Glucose-infused	0.318	0.356	0.354	0.309	0.274	0.027
	Control	0.245	0.252	0.235	0.230	0.328*	0.034
Non-lactating	Glucose-infused	0.375	0.437*	0.419	0.387	0.368	0.027
	Control	0.356	0.383	0.405	0.389	0.358	0.034
(b) Propionate infusion							
Lactating	Propionate-infused	0.291	0.561***	0.552***	0.511**	0.301	0.059
	Control	0.260	0.233	0.234	0.243	0.254	0.073
Non-lactating	Propionate-infused	0.430	0.600**	0.663***	0.503	0.446	0.059
	Control	0.418	0.353	0.307	0.318	0.308	0.073

Table 7. *Effects of infusion of either glucose or propionate on percentage hepatic extraction of lactate in lactating and non-lactating cows*

* $P < 0.02$ and ** $P < 0.001$ as compared with pre-infusion value.

Cow status	Treatment	Pre-infusion	Infusion			Post-infusion	Standard error of difference between means
			4.5 h	24 h	48 h		
(a) Glucose infusion							
Lactating	Glucose-infused	30.9	31.7	17.7	10.3*	27.4	7.4
	Control	22.9	32.3	24.7	27.4	21.4	9.0
Non-lactating	Glucose-infused	7.2	4.2	3.4	5.4	9.4	7.4
	Control	7.9	24.4	7.8	3.8	8.3	9.0
			0.5h	1.5h	3.0h		
(b) Propionate infusion							
Lactating	Propionate-infused	31.5	12.8*	10.6**	11.4**	26.1	4.6
	Control	23.8	25.2	29.7	20.6	19.5	5.6
Non-lactating	Propionate-infused	4.3	−1.4	−2.2	−3.8	0.1	4.6
	Control	4.0	5.8	2.7	4.2	5.8	5.6

observed in the control animals. By contrast, the propionate infusion produced marked increases in lactate concentration in both groups of cows (Table 6b).

Finally, the data in Table 7 demonstrate that in the lactating cows the decreases in hepatic lactate uptake elicited by both the glucose and propionate infusions involved significant decreases in the hepatic extraction of lactate. This implies that the decreases in lactate uptake in this group were not simply due to a decrease in the rate of arrival of lactate at the liver. In fact, the latter parameter remained essentially unchanged in magnitude during the glucose infusion and increased during the propionate infusion, in line with arterial lactate concentration, since blood flow rate was not affected by either infusion (results not shown).

The effect of the glucose and propionate infusions on pyruvate exchange across the liver was also monitored in a limited number of experiments. As Table 8 shows, in lactating cows both infusions caused the net hepatic uptake of pyruvate,

characteristic of the untreated animals, to be changed into an output. This change was in line with the effect of these treatments in decreasing hepatic uptake and extraction of lactate in this group (Tables 5 and 7). On the other hand, propionate infusion had little or no effect on pyruvate exchange across the liver in a non-lactating cow (Table 8b).

Earlier studies *in vitro* demonstrated that the uptake of lactate and pyruvate by perfused rat liver and by isolated sheep liver mitochondria was diminished in the presence of propionate (Smith & Osborne-White, 1971; Chan & Freedland, 1972). The present work now shows that a similar effect can occur in cows during propionate infusion *in vivo* (Tables 5 and 8). It is possible that the decrease in net hepatic uptake of lactate and pyruvate during the propionate infusion was not due to a simple decrease in uptake of these compounds, but rather to conversion of propionate, via phosphoenolpyruvate carboxykinase (EC 4.1.1.32) and pyruvate kinase (EC 2.7.1.40), to pyruvate and lactate, which were then released. However, Chan &

Table 8. *Effects of infusion of either glucose or propionate on net hepatic production of pyruvate in cows*
Production rates are expressed as mmol/min in whole blood. The values on each line are the results obtained for one animal. N.D., Not determined.

Cow status	Treatment	Pre-infusion	Infusion	
			24h	48h
(a) Glucose infusion				
	Lactating			
	Glucose-infused	-0.20	+0.12	+0.14
	Control	N.D.	N.D.	-0.14
3h				
(b) Propionate infusion				
	Lactating			
	Propionate-infused	-0.27		+0.34
	Control	-0.23		-0.12
Non-lactating				
	Propionate-infused	+0.12		+0.08
	Control	+0.27		-0.02

Table 9. *Effect of infusion of either glucose, propionate or lactate on net hepatic production of propionate in cows*
Hepatic production is expressed as mmol/min. * $P < 0.05$ and ** $P < 0.001$ as compared with pre-infusion value.

Cow status	Treatment	Pre-infusion	Infusion			Post-infusion	Standard error of difference between means
			4.5h	24h	48h		
(a) Glucose infusion							
	Lactating						
	Glucose-infused	-7.20	-6.80	-7.09	-6.91	-7.00	1.30
	Control	-10.06	-10.06	-7.48	-10.09	-6.96	1.59
Non-lactating							
	Glucose-infused	-4.78	-5.05	-5.09	-6.02	-5.85	1.30
	Control	-3.58	-5.67	-4.38	-4.43	-4.77	1.59
			0.5h	1.5h	3.0h		
(b) Propionate infusion							
	Lactating						
	Propionate-infused	-6.30	-12.28**	-12.45**	-13.41**	-7.90	1.02
	Control	-8.31	-7.26	-5.98	-7.13	-8.19	1.24
Non-lactating							
	Propionate-infused	-5.80	-11.74**	-11.70**	-13.50**	-4.61	1.02
	Control	-4.58	-1.85*	-2.01	-2.63	-3.97	1.24
			0.5h	1.5h	3.0h		
(c) Lactate infusion							
	Lactating						
	Lactate-infused	-6.78	-7.09	-6.38	-4.99	-6.73	1.22
	Control	-8.31	-6.55	-5.98	-7.13	-8.19	1.22

Freedland (1972) found little evidence for this in perfused rat liver.

It is apparent therefore that, in the lactating cows, an effect of the glucose and propionate infusions was to bring parameters of hepatic lactate and pyruvate metabolism more into line with those seen in untreated non-lactating cows, and to abolish net uptake of lactate and pyruvate by the splanchnic bed. By contrast, neither glucose, propionate nor lactate infusion elicited any decrease in hepatic propionate uptake in either groups of cows. This is shown in Table 9. The propionate infusion in fact resulted in an increase in hepatic uptake of propionate amounting to about 6–7 mmol of propionate/min in both groups of cows. These increases were statistically highly significant (Table 9). The glucose and lactate infusions had no effect on hepatic extraction of propionate. During the propionate infusion, however, hepatic extraction fell from 80–85% to 70%. None of the

infusions had any consistent effect on gut output of propionate (results not shown).

It is evident from consideration of Tables 3 and 9 that cessation of net uptake of lactate and pyruvate by the splanchnic bed of the lactating cows during the glucose and propionate infusions was accompanied by an increase in the rate of uptake of propionate by the liver relative to the rate of glucose output, i.e. by an increase in the magnitude of the hepatic (propionate uptake)/(glucose output) ratio.

Withdrawal of exogenous precursors

When lactating and non-lactating cows were deprived of food for 6 days, hepatic uptake of the exogenous precursor propionate ceased (Table 10d), since propionate was no longer being absorbed from the gut. At the same time hepatic glucose output declined to about 20% of the fed value in the two

Table 10. *Splanchnic metabolism of lactate and pyruvate and hepatic production of glucose and propionate in lactating and non-lactating cows before and during food deprivation*

Arterial concentrations are expressed as mM, production rates as mmol/min and hepatic extraction as a percentage.
 * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ as compared with fed value.

		Deprived of food					Standard error of difference between means
Cow status	Parameter	Fed	1 day	2 days	4 days	6 days	
(a) Lactate							
Lactating	Arterial concentration	0.32	0.37	0.32	0.45	0.57	0.15
	Gut production	1.33	0.66	2.20	−0.03*	0.12*	0.48
	Liver production	−2.54	−3.08	−3.45	−1.81	−2.18	1.20
	Hepatic extraction	26.3	42.0**	52.8***	35.6*	34.4	3.65
Non-lactating	Arterial concentration	0.46	0.28	0.25	0.33	0.32	0.15
	Gut production	1.56	1.26	0.38*	0.19*	0.27*	0.48
	Liver production	−0.92	−1.96	−1.92	−1.92	−1.49	1.20
	Hepatic extraction	9.3	40.4***	49.9***	47.1***	43.3***	3.65
(b) Pyruvate							
Lactating	Arterial concentration	0.019	0.018	0.018	0.024	0.033	0.015
	Gut production	0.058	0.120	0.040	0.055	0.015	0.057
	Liver production	−0.250	−0.265	−0.185	−0.180	−0.235	0.098
	Hepatic extraction	44.3	59.6*	58.3*	54.9	60.4*	5.04
Non-lactating	Arterial concentration	0.035	0.019	0.016	0.025	0.021	0.015
	Gut production	−0.043	0.070	0.076	0.040	0.045	0.057
	Liver production	0.135	−0.175*	−0.218**	−0.215**	−0.165*	0.098
	Hepatic extraction	−19.3	52.3***	71.6***	65.2***	66.3***	5.04
(c) Glucose							
All cows	Liver production	5.41	2.97	1.63**	1.14**	1.23**	1.13
(d) Propionate							
All cows	Liver production	−4.98	−0.91***	−0.45***	−0.05***	−0.02***	0.75

Table 11. *Effect of food deprivation on net uptake of lactate by the splanchnic bed in lactating and non-lactating cows*

Net uptake is expressed as mmol/min. The negative value indicates net output. Each value represents the algebraic sum of gut and liver production as given in Table 10.

Cow status	Fed	Deprived of food			
		1 day	2 days	4 days	6 days
Lactating	1.21	2.42	1.25	1.78	2.06
Non-lactating	-0.64	0.70	1.54	1.73	1.22

groups of cows taken together (Table 10c), and gut output of lactate plus pyruvate declined on average to 16% of the fed value (Table 10a). Despite these changes, hepatic uptake of lactate was maintained in the lactating cows at about the same level as in the fed state throughout food deprivation, while this parameter doubled in the non-lactating cows, although statistical significance was not achieved for the increase. Maintenance or increase in the rate of hepatic lactate uptake was achieved in the face of a substantial decline in the rate of arrival of lactate at the liver. Owing to the decline in gut output of lactate and the fall in blood flow rate (result not shown), the rate of arrival fell during food deprivation by 46% in the lactating cows and 64% in the non-

lactating cows. This situation is expressed by the fact that hepatic extraction of lactate increased significantly in both groups of cows.

Owing to the persistence of hepatic lactate uptake in the lactating cows, net uptake by the splanchnic bed of endogenously derived lactate was maintained or increased during food deprivation (Table 11). In the non-lactating cows there was a switch, already evident on day 1 of food deprivation, from net output to net uptake of lactate by the splanchnic bed (Table 11). This switch is consistent with the finding (MacDonald *et al.*, 1976; Ruderman *et al.*, 1977) that there is a release of lactate from rat skeletal muscle during food deprivation. However, Bergman *et al.* (1974) failed to observe any increase in turnover within the Cori cycle, as measured by isotope dilution, when non-lactating sheep were deprived of food. The effects of food deprivation on pyruvate metabolism (Table 10b) mirrored in essence those of lactate metabolism. A switch in liver pyruvate production from net output to net uptake had already occurred in the non-lactating cows after only 1 day of food deprivation. In both groups of cows hepatic pyruvate extraction increased significantly during food deprivation.

The changes in lactate and pyruvate exchange during food deprivation were accompanied by a decrease in the hepatic (propionate uptake)/(glucose

output) ratio (Table 10). Further evidence is thus provided in support of an approximate inverse relationship between this ratio and the utilization of endogenous lactate and pyruvate by the splanchnic bed.

Conclusions

General points. The infusion experiments demonstrated that when there was a surfeit of glucose, or of exogenous glucose precursor in the form of propionate, hepatic uptake of lactate and pyruvate declined, and as a result the uptake of these latter compounds by the splanchnic bed ceased. Conversely, when there was a deficit of exogenous precursor, as occurred during food deprivation, uptake of lactate and pyruvate by the liver and the splanchnic bed was either maintained or increased. On the other hand, there appeared to be no rapid mechanism for regulating uptake of propionate by the liver, even when this process might seem excessive. Thus, during the glucose infusion, liver uptake of propionate at 4.5 and 24 h was more than sufficient to account for the whole of the hepatic output of glucose in the lactating cows (cf. Tables 3 and 9).

During the glucose infusion, decrease in the uptake of lactate by the liver must have been an initiating factor in the termination of lactate uptake by the splanchnic bed in the lactating cows, since hepatic extraction of lactate decreased, even though the rate of arrival of lactate at the liver remained constant. It seems unlikely that the decrease in liver lactate uptake could have been elicited solely by changes in the circulating concentrations of either insulin or glucose, since these concentrations were not greatly altered at 24 and 48 h of glucose infusion, at least (Table 4). The fact that the arterial lactate concentration did not increase markedly in the lactating cows in this experiment (Table 6) suggests either that recycling of lactate from peripheral tissues decreased, or that there was an increase in lactate utilization for milk production, which increased by some 6% during the infusion. Decrease in lactate recycling would be consistent with the observation of Bartley & Black (1966) that the contribution of glucose to CO_2 formation increased in lactating cows during glucose infusion.

Regulation of hepatic uptake of lactate and pyruvate. The present study offers no direct evidence to explain how liver uptake and extraction of lactate and pyruvate might be regulated. Nevertheless, the apparent relationship between these parameters and the ratio across the liver of propionate uptake to glucose output suggests that some characteristic of the gluconeogenic pathway between propionate and glucose could play a role in this respect. Such a mechanism could help to explain how uptake of lactate and pyruvate by the liver is integrated with

the need of the body as a whole to recycle glucose carbon when the supply of exogenous precursor is limited in relation to the prevailing demand for glucose. Comparison of the present study with earlier work from this laboratory indicates that one of the characteristics that could be of importance is the steady-state concentration in the liver of intermediates or precursors of the tricarboxylic acid cycle. The concentrations of several of these compounds were found to vary in relation to physiological and nutritional status. Among the compounds whose concentrations varied in this way were citrate, 2-oxoglutarate, oxaloacetate and pyruvate (Baird & Heitzman, 1970; Baird *et al.*, 1972, 1979; Treacher *et al.*, 1976). In general, the concentrations of these compounds were low in situations where the hepatic uptake of lactate and pyruvate was high in the present study, and vice versa. The effect of propionate infusion on the concentration of the compounds has not been monitored. However, the fact that in the lactating cows in the present study propionate uptake by the liver increased while glucose output remained essentially unchanged (Tables 3 and 9) indicates that there may have been an increase in concentration in this situation as well. Reciprocity between the hepatic concentrations of intermediates or precursors of the tricarboxylic acid cycle on the one hand and the hepatic uptake of lactate and pyruvate on the other suggests a functional linkage between the two sets of parameters. If this reciprocity also applied to the hepatic concentrations of succinoyl-CoA, an intermediate of the cycle, and methylmalonyl-CoA, a precursor, both of which are on the pathway of propionate metabolism, then control of the uptake of lactate and pyruvate might be achieved by allosteric regulation of pyruvate carboxylase activity, since both succinoyl-CoA and methylmalonyl-CoA have been shown to inhibit this enzyme in other species (Barritt *et al.*, 1976). Control in this manner would emphasize an anaplerotic role for the assimilated lactate and pyruvate. Against this hypothesis must be set the fact that propionyl-CoA, whose concentration might also vary in a reciprocal manner with hepatic uptake of lactate and pyruvate, is a known activator of pyruvate carboxylase (Barritt *et al.*, 1976).

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