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Anti-ketogenic Effect of Glucose in the Lactating Cow Deprived of Food

By ROYCE J. TREACHER, G. DAVID BAIRD and JANICE L. YOUNG
Agricultural Research Council Institute for Research on Animal Diseases, Compton, Berks. RG16 0NN, U.K.

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1. The aim of this study was to determine the effect of a constant infusion of glucose on the ketosis that is observed when dairy cows are deprived of food in early lactation. 2. Cows in early lactation were first deprived of food for 4 days (96h) to induce a 'fasting ketosis'. Glucose was then infused intravenously at a constant rate of 0.75g/min for 48h while deprivation of food was maintained. At the end of this 48h period, blood and liver ketone-body concentrations had decreased to values well below those found in healthy fed cows. 3. On the assumption that the anti-ketogenic effect of glucose was mainly due to suppression of hepatic ketogenesis, it was concluded that two anti-ketogenic mechanisms had been identified. These were (*a*) a decrease in the availability of free fatty acids for hepatic oxidation, and (*b*) anti-ketogenic changes within the liver itself. 4. These latter anti-ketogenic changes were twofold. The first was a major increase in the hepatic concentrations of citrate and 2-oxoglutarate. The second was an increase in the degree of oxidation of the hepatic cytosol. It was proposed that both these intrahepatic changes might indicate an augmentation of the quantity of oxaloacetate available for condensation with acetyl-CoA derived from fat oxidation. 5. Hepatic glycerol 1-phosphate concentration fell substantially after glucose infusion. 6. Glucose infusion into fed cows produced qualitatively similar effects to those observed in the unfed cows. However, blood and liver ketone-body concentrations were not decreased to the same extent in the fed cows as in the unfed cows.

Depriving dairy cows of food in early lactation often leads to a severe ketosis with greatly increased blood concentrations of ketone bodies and free fatty acids and decreased blood glucose concentrations (Baird *et al.*, 1972). The unfed lactating dairy cow therefore appears to offer an appropriate model in which to study the effect of anti-ketogenic agents. If the deprivation of food is maintained while the effect of the anti-ketogenic agent is being monitored, then any ambiguities in interpretation of the data as a result of variation in food intake are eliminated. This is not necessarily the case when the effects of anti-ketogenic agents are being examined in cases of bovine ketosis (spontaneously arising ketosis in dairy cows in early lactation), since cows suffering from this latter condition are usually still eating, and appetite tends to improve as a result of administering the anti-ketogenic agent (e.g. Baird & Heitzman, 1971).

In the fed ruminant there are two sites of ketogenesis, the liver and the rumen epithelium (Bergman, 1971; Baird *et al.*, 1975). However, in the unfed ruminant ketogenesis from the rumen epithelium ceases and the liver becomes the sole organ of ketogenesis, producing large quantities of ketone bodies from fatty acid oxidation (Katz & Bergman, 1969; Bergman, 1971; G. D. Baird, H. W. Symonds & I. M. Reid, unpublished work). Any anti-ketogenic effect observed in such animals is likely therefore to involve a decrease in the rate of hepatic ketogenesis from fatty acid.

It is well known that parenterally administered glucose has an anti-ketogenic effect in cattle (see, e.g., Kronfeld, 1972). In the present study, glucose has been infused into unfed lactating dairy cows to attempt to determine the mechanism by which it might suppress hepatic ketogenesis in these animals. Glucose has also been infused into fed lactating cows to determine whether the biochemical changes elicited by infusion of this compound into unfed cows also occur in the fed state.

The anti-ketogenic effect of glucose has been assessed in the light of the biochemical changes occurring in the blood and liver during infusion. It appears that the effect is due to at least two factors: (i) decreasing the supply of free fatty acids to the liver and (ii) eliciting metabolic changes within the liver itself that are likely to lead to a decrease in the rate of hepatic ketone-body formation.

Experimental

Materials

Substrates and enzymes for metabolite concentration determinations were from Boehringer Corp. (London), Lewes, Sussex, U.K. Other chemicals were of analytical grade. Double-distilled water, the second distillation being from glass, was used throughout.

Animals

Thirteen lactating dairy cows were used, comprising Ayrshire, Friesian and Friesian–Ayrshire animals. All the cows were in early lactation (15–60 days after calving) and had been through at least one previous lactation. When being fed the animals received a daily maintenance ration consisting of 9kg of barley straw plus 1kg of dairy concentrate. The concentrate contained 16% (w/w) crude protein and 11.5MJ of metabolizable energy/kg wet wt. Additional concentrate was then fed at the daily rate of 0.4kg/kg of milk produced. The cows were divided into three groups. These groups were a glucose-treated unfed group (four cows), a glucose-treated fed group (five cows) and a water-treated fed group (four cows). This latter group represented the control group (see below).

Methods

Experimental procedure. In an earlier study (Baird *et al.*, 1972) dairy cows in early lactation were deprived of food for 6 days (144h) and subjected to liver biopsy at the end of the period. Hepatic metabolite concentrations were then determined in the freeze-clamped liver-biopsy samples. During this study (Baird *et al.*, 1972) it was observed that the changes in blood metabolite concentration that were characteristic of 'fasting ketosis' had already occurred after 4 days (96h) of deprivation of food. In order to determine the effects of glucose on 'fasting ketosis' in the present work it was deemed therefore appropriate to start the glucose infusion after 4 days of deprivation of food and to maintain the infusion until the cow had been deprived of food for a further 2 days (48h). The effect of the glucose infusion on blood metabolite concentrations characteristic of the unfed animal could then be monitored over this 2-day period. Further, the steady-state metabolite concentrations in liver biopsy samples taken at the end of this period could be compared directly with those observed in the earlier study in lactating cows that were deprived of food for 6 days, but did not receive a glucose infusion (Baird *et al.*, 1972). It was also decided in the present study to complement these experiments with an examination of the effect of a similar glucose infusion on liver and blood metabolite concentrations in fed lactating cows. Finally, hepatic metabolite concentrations were also determined in fed lactating cows that had received a water infusion under conditions comparable with those used for glucose infusion. This group acted as a control for any effects that the infusion and liver-biopsy procedures may have had on hepatic metabolite concentrations.

In the glucose-treated unfed group therefore the cows were deprived of food for a total of 144h as described by Baird *et al.* (1972). After 96h of deprivation of food, infusion of a 50% (w/v) solution of glucose was begun at a constant rate of 1.5ml/min (0.75g of glucose/min), and this infusion was then continued for the remaining 48h of deprivation of food. Liver biopsy was performed at the end of this final 48h period, while the glucose infusion was being maintained and before feeding had recommenced. Over the 48h infusion period approx. 2150g of glucose was delivered in about 4.3 litres of solution to each animal. In the glucose-treated fed group the infusion and liver biopsy procedures were the same as in the glucose-treated unfed group. However, the cows were fed normally. Finally, in the control group the infusion and biopsy procedures were again similar, except that in this case water was infused instead of the glucose solution. Again the animals were fed normally. In view of the fact that the jugular blood flow rate must have been some 2000 times the water infusion rate, the water infusion cannot have affected blood composition or caused haemolysis to any significant degree. It was considered that water was the most appropriate control fluid to infuse since the glucose was dissolved in water only. However, some preliminary experiments were carried out with a control infusion of 0.9% NaCl. Hepatic metabolite concentrations in biopsy samples taken at the end of these infusions showed no difference from those taken at the end of water infusions.

Glucose infusion. Glucose solution or water was infused into each animal via a jugular vein. The method of infusion was that described by Treacher (1973). In this method a Harvard pump (Harvard Apparatus, Millis, MA, U.S.A.) is used, the pump being inserted into a pocket of a harness carried on the back of the animal. The harness was strapped on to the cow some 48h before infusion was started.

Liver tissue. This was obtained by biopsy via a laparotomy incision. It was freeze-clamped immediately and subsequently extracted with 30% (w/v) $HClO_4$, as described by Baird *et al.* (1968). Local anaesthesia was performed with an adrenaline-free anaesthetic (2% Xylocaine; Astra Chemicals, Watford, Herts., U.K.). Only four of the cows in the glucose-treated fed group were subjected to liver biopsy.

Blood. In the glucose-treated unfed group blood samples were collected immediately before the infusion, after 24h of infusion, and after 48h of infusion immediately before liver biopsy. In two cows in this group further samples were taken at 4, 6, 8 and 10h

after starting the infusion. In the glucose-treated fed group blood samples were only taken as a routine at zero time and after 48h of infusion. However, in two cows in this group samples were also taken at 4, 6, 8, 10 and 24h after starting infusion. The blood samples were obtained from the jugular vein that was not being used for the infusion, and were collected in 6% (w/v) $HClO_4$ or in heparin for metabolite assays (Baird & Heitzman, 1970).

Metabolite concentrations in liver and blood. The following compounds were assayed by the methods used by Baird & Heitzman (1970): D(−)-3-hydroxybutyrate; acetoacetate; lactate; pyruvate; citrate; 2-oxoglutarate; malate; phosphoenolpyruvate; 2-phosphoglycerate; 3-phosphoglycerate; glycerol 1-phosphate; glycogen. Glucose was assayed by the method of Slein (1963) and glycerol by the method of Wieland (1963). Dihydroxyacetone phosphate, glyceraldehyde phosphate and fructose 1,6-bisphosphate were determined by the method of Bücher & Hohorst (1963). Plasma free-fatty acid concentrations were determined by using the test kit supplied by Boehringer. All the hepatic metabolite concentrations were determined in neutralized $HClO_4$ extracts of freeze-clamped liver. All the blood metabolite concentrations were determined in neutralized-$HClO_4$ extracts of blood, except for that for free fatty acids, which was determined in heparinized blood plasma.

Statistics. Probability values (P) were obtained by Student's t test. Daily observations of changes in blood metabolite concentrations during glucose infusion were analysed by analysis of variance (see Steel & Torrie, 1960).

Results

Blood metabolite concentrations

Table 1 shows the blood metabolite concentration changes that were observed when glucose was infused into the unfed cows. Immediately before starting the infusion, i.e. after 4 days' deprivation of food, the concentrations of the metabolites were characteristic of those found in 'fasting ketosis', including high concentrations of ketone bodies, free fatty acids and glycerol, and low concentrations of glucose, citrate and pyruvate. (Typical values for fed cows in early lactation are given in the 'Before infusion' column in Table 2; see below.) After the glucose infusion had been in progress for 24h, the concentration of the ketone bodies had fallen to values lower than those seen in normal fed cows (e.g. Table 2), whereas that of glucose had increased some 4-fold to a concentration some 2–3 times the normal for a fed animal. Over this period the concentration of free fatty acid had also fallen, but was still 4–5 times that observed in the normal fed cow in early lactation. At the end of 48h of infusion ketone bodies were hardly detectable in the blood, but the concentration of free fatty acid was now some 2–3 times greater than normal. The glucose concentration had fallen somewhat, but was still 2 times greater than normal. The changes in the concentrations of the other blood constituents measured during the glucose infusion were of smaller size, but noteworthy were the increase in the [hydroxybutyrate]/[acetoacetate] ratio, the decrease in the [lactate]/[pyruvate] ratio and the increase in citrate and pyruvate concentrations.

Table 1. *Effect of glucose infusion on blood metabolite concentrations in unfed lactating cows*

Concentrations are expressed as μmol/ml of whole blood, except for that for free fatty acids, which is expressed as μequiv./ml of plasma. The values are for four animals and were examined by analysis of variance. Significances in relation to the value before infusion are: $*P<0.05$; $**P<0.01$; $***P<0.001$.

	Concentration or ratio			
	Before infusion	Infusion in progress for 24h	Infusion in progress for 48h	S.E.M.†
3-Hydroxybutyrate	5.31	0.18***	0.06***	0.67
Acetoacetate	1.250	0.012***	0.003***	0.15
Lactate	0.70	1.17	1.17	0.22
Pyruvate	0.032	0.088*	0.092*	0.022
Citrate	0.038	0.083*	0.092*	0.016
Glucose	1.40	6.93***	5.85**	0.91
Free fatty acids	5.50	2.00***	1.26***	0.57
Glycerol	0.152	0.107	0.083*	0.026
[3-Hydroxybutyrate]/[acetoacetate]	4.3	15.4	23.6*	7.3
[Lactate]/[pyruvate]	22.2	14.2*	12.8*	2.9

† Standard error of difference between any two means.

Table 2. *Effect of glucose infusion on blood metabolite concentrations in fed lactating cows*

Concentrations are expressed as μmol/ml of whole blood, except for that for free fatty acids, which is expressed as μequiv./ml of plasma. The values are for five animals (four in the case of free fatty acids) and were examined by analysis of variance. Significant differences in relation to the value before infusion are: $*P<0.05$; $**P<0.01$.

	Concentration or ratio		
	Before infusion	Infusion in progress for 48 h	S.E.M.†
3-Hydroxybutyrate	0.46	0.26*	0.07
Acetoacetate	0.030	0.007	0.009
Lactate	0.50	0.72	0.10
Pyruvate	0.048	0.058*	0.003
Citrate	0.138	0.082**	0.012
Glucose	2.57	3.04	0.24
Free fatty acids	0.46	0.14*	0.07
[3-Hydroxybutyrate]/[acetoacetate]	19.4	37.8**	3.4
[Lactate]/[pyruvate]	10.7	12.2	1.4

† Standard error of difference between any two means.

In two of the unfed cows the changes in the blood concentrations of 3-hydroxybutyrate, free fatty acid, glycerol and glucose were measured at intervals during the first 10h of glucose infusion as well, and these values are plotted in Fig. 1(*a*). Fig. 1(*a*) shows that substantial changes in the concentrations of these four components had already taken place by the time the infusion had been in progress for 4h. Thus, at this time, ketone-body, free fatty acid and glycerol concentrations had fallen to 55%, 45% and 50% respectively, and the glucose concentration had risen to 400% of the corresponding pre-infusion value. From 4h to 24h of the glucose infusion, however, the concentrations of free fatty acid and glycerol tended to remain constant or to decrease slowly. In contrast, that of hydroxybutyrate continued to decrease rapidly. Clearly, there was marked glucose intolerance in the unfed cows, but this did not prevent the infused glucose having profound effects on concentrations of blood metabolites.

The changes in concentration of blood constituents occurring when glucose was infused into well-fed cows are shown in Table 2. In general these changes were of much smaller magnitude than those occurring in the unfed cows. Thus 3-hydroxybutyrate concentration had only decreased by some 40% after 48h of infusion, and the concentration at this time was higher than that in the unfed cows after 24h of infusion. After 48h of glucose infusion the total ketone body concentration in the fed cows was 4 times that in the unfed cows at the corresponding time (0.262 and 0.062mM respectively; $P<0.001$ for difference of means). In percentage terms, the overall fall in free-fatty acid concentration was similar to that in the unfed cows, but the final concentration reached was of course some 10 times lower. The fed cows showed a much greater degree of glucose tolerance, the concentration of glucose after 48h of infusion being only 18% higher than that before infusion. Fig. 1(*b*) plots the changes in the blood concentrations of 3-hydroxybutyrate, free fatty acids, glucose and glycerol in two of the fed cows over the first 10h of glucose infusion, as well as after 24 and 48h of infusion. As Fig. 1(*b*) shows, there was an initial rise in blood glucose concentration during the infusion, but the maximum concentration reached was only 4.5mM (i.e. 190% of the pre-infusion value) and by 10h the glucose concentration was declining. By this time the free-fatty acid and hydroxybutyrate concentrations had fallen to 33% and 72% respectively of the pre-infusion values. There was little change in glycerol concentration during the infusion.

Milk yield

Fig. 2 shows the average daily milk yields that were observed in the various groups of cows in this study. In both groups of fed cows the insertion of the infusion catheter into the jugular vein, and the accompanying handling of the animals, appears to have caused a slight fall in milk yield. In the glucose-treated fed group milk yield rose once more during the second half of the infusion, presumably as a result of the availability of exogenous glucose. Fig. 2 also shows that infusion of glucose into the unfed cows caused the decline in milk yield due to the deprivation of food (Baird *et al.*, 1972) to be arrested. However, in neither glucose-treated group did the glucose infusion cause any major increase in milk yield over the 48h period.

Effect of glucose infusion on hepatic metabolite concentrations

The metabolite concentrations found in the liver biopsy samples taken from the three groups of cows

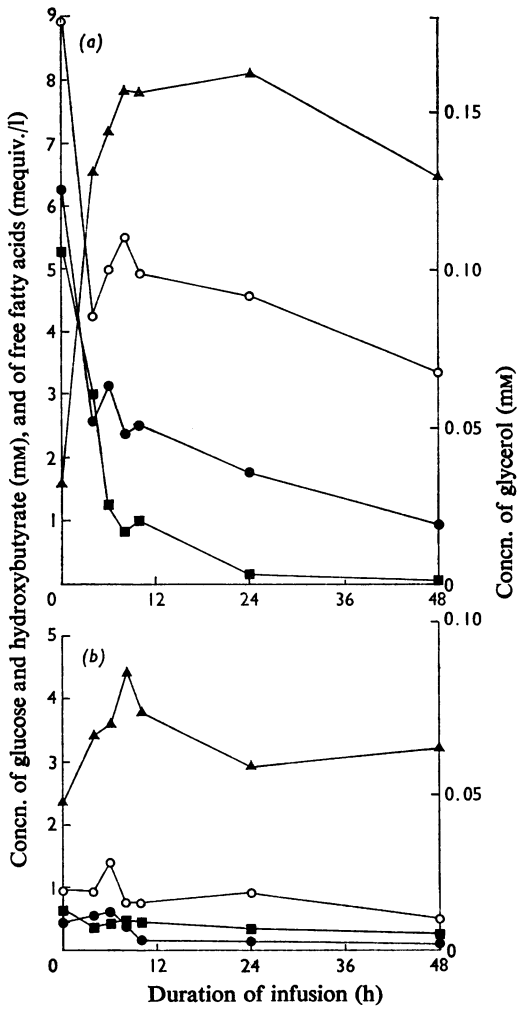

Fig. 1. *Effect of glucose infusion over a 48 h period on blood concentrations of glucose* (▲), *3-hydroxybutyrate* (■), *free fatty acids* (●) *and glycerol* (○) *in unfed lactating cows* (*a*) *and in well-fed lactating cows* (*b*)

In both (*a*) and (*b*) the values are in each case the means for two animals.

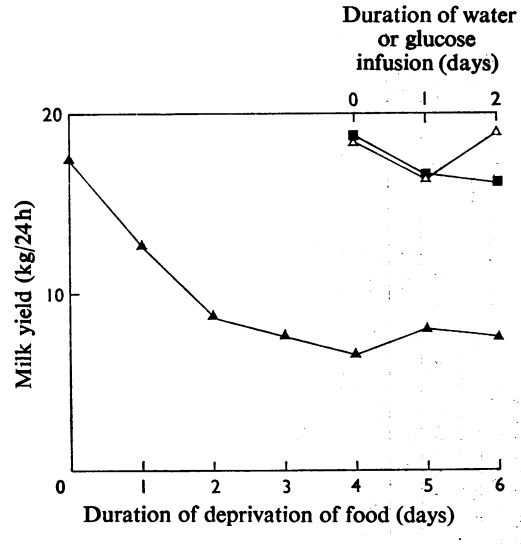

Fig. 2. *Effect of glucose infusion on milk yields*

Values are plotted for the three groups of cows: lactating cows deprived of food for 6 days (144 h) and receiving a glucose infusion during the last 48 h of the food-deprivation period (▲); well-fed lactating cows receiving a glucose infusion for 48 h (△); well-fed lactating cows receiving a water infusion for 48 h (control group) (■). Values are means for all the animals in each group.

are listed in Table 3. For comparison the hepatic metabolite concentrations found in untreated unfed lactating cows in the earlier study of Baird *et al.* (1972) are also included in the Table.

Baird *et al.* (1972) found that, besides causing a 5-fold increase in hepatic ketone-body concentrations, depriving lactating cows of food led to apparent decreases in the hepatic concentrations of all the assayed metabolites, except malate, that were directly involved as intermediates in either the tricarboxylic acid cycle or the Embden–Meyerhof pathway. Statistical significance for these decreases did not occur in every instance, however. Besides these changes there was also a marked increase in the degree of cytosolic reduction.

Table 3 shows that administration of glucose to the unfed cows reversed most of the food-deprivation-induced concentration changes enumerated above. The hepatic ketone-body concentration in the glucose-treated unfed cows was found to be only some 3% of that in the untreated unfed cows, and only some 14% of that in the fed group of control cows. Further, those intermediates whose hepatic concentrations were observed in the earlier work to be decreased by food-deprivation were in general present in higher concentrations in the glucose-treated unfed cows than in the untreated unfed cows. However, glucose administration to the unfed cows caused a much greater proportional increase in the hepatic concentrations of intermediates of the tricarboxylic acid cycle than of intermediates of the Embden–Meyerhof pathway. Thus the percentage increases were much greater for citrate and 2-oxoglutarate than for phosphoenolpyruvate and the phosphoglycerates. Also hepatic glycogen concentration was markedly higher in the glucose-treated unfed cows than in the untreated unfed cows of Baird *et al.* (1972). The glucose treatment of the unfed cows

Table 3. *Effect of glucose infusion on hepatic metabolite concentrations in unfed and in fed lactating cows*

The data for the control group of unfed cows are taken from Baird *et al.* (1972). These cows did not receive an infusion of any sort. The concentrations of the metabolites are expressed in μmol/g wet wt. of liver, except for that of glycogen, which is expressed as μmol of glucose equivalent/g wet wt. of liver. The values are means ±S.D. For the glucose-treated fed cows the numbers of cows examined are given in parentheses. The significances of the differences between means were determined by Student's *t* test. N.S., not significant ($P>0.05$); N.D., not determined.

Metabolite	Concentration or ratio: Unfed cows +glucose (UG; no. of cows=4)	Unfed cows (control; U; no. of cows=5)	Fed cows+glucose (FG)	Fed cows+water (control; F; no. of cows=4)	Comparison of values for group UG with group U: % of value for U	Comparison of values for group UG with group U: Significance of difference between means	Comparison of values for group UG with group F: % of value for F	Comparison of values for group UG with group F: Significance of difference between means	Comparison of values for group FG with group F: % of value for F	Comparison of values for group FG with group F: Significance of difference between means
3-Hydroxybutyrate	0.067±0.005	3.00 ±1.98	0.32 ±0.08 (4)	0.67 ±0.27	2	$P<0.05$	10	$P<0.01$	48	$P<0.05$
Acetoacetate	0.035±0.003	0.63 ±0.50	0.029±0.009 (3)	0.048±0.006	5	$P<0.05$	73	$P<0.01$	60	$P<0.05$
Lactate	0.39 ±0.06	1.02 ±0.57	0.56 ±0.17 (4)	0.33 ±0.07	38	N.S.	118	N.S.	170	$P<0.05$
Pyruvate	0.041±0.004	0.024±0.016	0.054±0.018 (4)	0.026±0.004	171	N.S.	158	$P<0.01$	208	$P<0.05$
Citrate	0.87 ±0.64	0.075±0.027	0.62 ±0.08 (4)	0.34 ±0.09	1160	$P<0.05$	256	N.S.	182	$P<0.01$
2-Oxoglutarate	0.43 ±0.23	0.083±0.075	0.43 ±0.04 (4)	0.26 ±0.06	518	$P<0.02$	166	N.S.	166	$P<0.01$
Malate	0.98 ±0.87	0.75 ±0.45	0.69 ±0.62 (4)	0.60 ±0.18	131	N.S.	163	N.S.	115	N.S.
Phosphoenolpyruvate	0.062±0.008	0.042±0.039	0.10 ±0.05 (3)	0.084±0.027	148	N.S.	74	N.S.	119	N.S.
2-Phosphoglycerate	0.024±0.009	0.018±0.012	0.041±0.009 (3)	0.033±0.012	133	N.S.	73	N.S.	124	N.S.
3-Phosphoglycerate	0.17 ±0.04	0.099±0.097	0.26 ±0.12 (3)	0.26 ±0.05	172	N.S.	65	$P<0.05$	100	N.S.
Glycerol 1-phosphate	0.061±0.031	0.48 ±0.15	0.15 ±0.06 (4)	0.24 ±0.05	13	$P<0.001$	25	$P<0.001$	63	$P<0.05$
Dihydroxyacetone phosphate	0.025±0.005	N.D.	0.038±0.007 (4)	0.034±0.007	—	—	74	N.S.	112	N.S.
Glyceraldehyde phosphate	0.025±0.009	N.D.	0.024±0.017 (4)	0.017±0.006	—	—	147	N.S.	141	N.S.
Fructose bisphosphate	0.017±0.005	N.D.	0.022±0.010 (4)	0.025±0.007	—	—	68	N.S.	88	N.S.
Glucose	5.55 ±1.57	3.23 ±1.31	4.47 ±1.11 (3)	3.79 ±0.88	172	$P<0.05$	146	N.S.	118	N.S.
Glycogen	344±94	33±41	324±2 (3)	143±36	1040	$P<0.001$	240	$P<0.01$	226	$P<0.001$
[3-Hydroxybutyrate]/[acetoacetate]	2.0 ±0.1	4.6 (mean)	10.9 ±5.5 (3)	13.0 ±4.0	44	—	15	$P<0.01$	84	N.S.
[Lactate]/[pyruvate]	9.6 ±1.4	42.5 (mean)	10.0 ±3.1 (4)	13.0 ±4.2	23	—	74	N.S.	77	N.S.
[Glycerol 1-phosphate]/[dihydroxyacetone phosphate]	2.6 ±1.5	N.D.	3.9 ±1.0 (4)	7.6 ±2.8	—	—	34	$P<0.02$	51	$P<0.05$

in fact raised the hepatic concentrations of citrate, 2-oxoglutarate and glycogen to levels that were higher even than those found in the control group of fed cows. On the other hand, glucose administration to the unfed cows failed to raise the concentrations of phosphoenolpyruvate and the phosphoglycerates up to the values found in the fed control group.

Glucose treatment of the unfed cows also led to a re-oxidation of the hepatic cytosol, as shown by the decrease in the [lactate]/[pyruvate] ratio. The glucose-induced decrease in the [glycerol 1-phosphate]/[dihydroxyacetone phosphate] ratio was associated with a very marked decrease in glycerol 1-phosphate concentration. As can be seen from Table 3, the concentration of this latter compound in the glucose-treated unfed cows was one-eighth that in the untreated unfed cows and one-quarter that in the fed control cows.

Table 3 also shows that glucose administration to the fed cows produced effects that were qualitatively similar to those previously seen in the glucose-treated unfed cows, since the hepatic concentrations of citrate, 2-oxoglutarate and glycogen were all higher in the glucose-treated fed cows than in the water-treated fed group, whereas the hepatic concentration of the ketone bodies was lower. Further, the glucose infusion decreased the [glycerol 1-phosphate]/[dihydroxyacetone phosphate] ratio in the fed cows, but had little or no effect on the hepatic concentration of phosphoenolpyruvate and the phosphoglycerates.

Discussion

The rate of glucose infusion used in the present work (0.75g/min) approaches the rate of hepatic glucogenesis that is observed in lactating Friesian cows in the fed state, and is greater than that observed in such cows after 4 days' deprivation of food (about 1.0 and 0.4g of glucose/min respectively; Baird *et al.* 1975; G. D. Baird, I. M. Reid & H. W. Symonds, unpublished work). It is likely therefore that the glucose infusions caused substantial falls in endogenous glucose entry rate in both the unfed and fed groups of cows (see Annison & White, 1961; West & Passey, 1967; Judson & Leng, 1973; Thompson *et al.*, 1975). The glucose infusions are also likely to have elicited increased insulin production, which may in turn have contributed to the metabolic effects of the infusions.

The almost total elimination of ketone bodies from the blood of the unfed cows after 48h of glucose infusion was probably due to a large extent to suppression of hepatic ketogenesis (see Bergman & Kon, 1964). Concomitant facilitation of ketone-body utilization by extrahepatic tissues cannot be excluded, but no data exist on this point.

The rapid decline in the concentrations of the circulating free fatty acids that was immediately

evoked by the glucose infusion must have been a major factor in contributing to the decrease in hepatic ketogenesis. Thus the decline in free-fatty acid concentration must have been due to a partial reversal of the release of free fatty acid from adipose tissue (see West & Passey, 1967), so that less free fatty acid was available for hepatic uptake and oxidation. However, blood ketone-body concentrations continued to fall after the rapid decline in blood free-fatty concentration had been arrested (Fig. 1*a*), suggesting that hepatic ketogenesis was still continuing to decrease even though the supply of free fatty acids was no longer declining. Some of the fall in circulating ketone-body concentrations could have been due to their adjustment, by peripheral utilization, to the new, and lower, rate of hepatic ketogenesis. Nevertheless, the fact remains that after 24h of glucose infusion the blood ketone-body concentration in the glucose-treated unfed cows was only 39% of that found in untreated fed cows, even though the blood free-fatty acid concentration was still 435% of that in untreated fed animals (Table 1 and the 'Before infusion' column in Table 2). These considerations suggest therefore that the glucose infusion must also have mediated some anti-ketogenic mechanism within the liver itself.

Hepatic metabolite concentrations

The large increase in the hepatic concentrations of citrate and 2-oxoglutarate that were elicited by the glucose infusion in both the unfed and the fed cows are reminiscent of similar increases in the concentrations of these compounds, and of oxaloacetate and malate, elicited by administration of the glucocorticoid Voren (dexamethasone-21-pyridine 4-carboxylate; Boehringer, Ingelheim, W. Germany) to fed healthy cows and to cows suffering from bovine ketosis (Baird & Heitzman, 1970, 1971). Voren was found to cause a simultaneous decrease in the cytoplasmic activity of phosphoenolpyruvate carboxykinase (EC 4.1.1.32) (Baird & Heitzman, 1970, 1971; Heitzman *et al.*, 1972), and it was concluded that the rises in the concentrations of the intermediates of the tricarboxylic acid cycle were a consequence of the decrease in phosphoenolpyruvate carboxykinase activity. It is possible that the glucose-induced increases in hepatic citrate and 2-oxoglutarate concentration in the present work could also be associated with a decrease in the activity of this enzyme. The fact that the malate concentration failed to increase is not necessarily inconsistent with this hypothesis, since any such increase might be opposed by the marked change in cytoplasmic redox state associated with the glucose infusion.

If glucose infusion does indeed cause a decrease in phosphoenolpyruvate carboxykinase activity, this could represent the means by which application of a glucose load decreases endogenous glucose entry rate in ruminants (Thompson *et al.*, 1975). Glucose administration decreases phosphoenolpyruvate carboxykinase activity in rats deprived of food (Shrago *et al.*, 1967), whereas the activity of the enzyme in perfused rat liver is inversely related to the concentration of circulating glucose (Moreno *et al.*, 1975).

The marked increase in the degree of oxidation of the liver cytoplasm that occurred when glucose was administered to the unfed cows was presumably due to the decrease in the availability of free fatty acids for oxidation in the liver (see, e.g., Wieland, 1968), a factor that must have greatly outweighed any increase in reducing equivalents due to decreased gluconeogenesis (Blackshear *et al.*, 1975). A difficulty in interpreting the cytosolic redox data for the glucose-treated unfed cows is that the [lactate]/[pyruvate] and [glycerol 1-phosphate]/[dihydroxyacetone phosphate] ratios appear to be indicating different redox states. Although the former ratio indicates that the cytosol in these cows was as oxidized as that of the fed control cows, the latter ratio suggests that the cytosol was more oxidized than in the control cows. Thus in the livers of the control fed cows the value of the ratio:

$$\frac{[\text{lactate}]}{[\text{pyruvate}]} \times \frac{[\text{dihydroxyacetone phosphate}]}{[\text{glycerol 1-phosphate}]}$$

is 1.7, which is identical to the value of the ratio of the mass-action equilibrium constants for the glycerol phosphate dehydrogenase (EC 1.1.1.8) and lactate dehydrogenase (EC 1.1.1.27) reactions (Hohorst *et al.*, 1961). However, in the glucose-treated unfed cows the value is 3.7. An explanation for this could be that in these latter animals the glycerol 1-phosphate concentration was lower than would be expected from the cytosolic redox state, because it was being rapidly utilized for esterification of long-chain acyl-CoA.

Anti-ketogenic action of glucose

The present study shows that infused glucose probably exerts an anti-ketogenic action in the unfed cows by at least two mechanisms. These are (i) eliciting a substantial decline in the concentration of circulating free fatty acids, and (ii) eliciting metabolic alterations within the liver that are likely to decrease the production of ketone bodies from those fatty acids that do reach the liver. Mechanism (i) has already been dealt with above and this discussion will therefore be confined to mechanism (ii).

One anti-ketogenic mechanism that may occur as a result of the glucose infusion has already been touched upon. This is the possibility that the rate of triglyceride synthesis might be stimulated. Fritz (1961) and McGarry & Foster (1972) have emphasized the importance of increased triglyceride synthesis as an anti-ketogenic mechanism in which free fatty acid

is diverted from the oxidative pathway. In this context McGarry & Foster (1972) suggested that the ability of insulin to decrease hepatic ketogenesis in unfed rats even when the supply of free fatty acids was artificially maintained (Bieberdorf *et al.*, 1970) could be due to an insulin-stimulated increase in triglyceride formation.

A second possibility is that the glucose infusion may have increased the hepatic concentration of oxaloacetate. Oxaloacetate concentrations were not measured in this study. However, in several earlier studies changes in the hepatic concentration of citrate have been found to be accompanied by corresponding changes in oxaloacetate concentration in the same direction (Baird & Heitzman, 1971; Baird *et al.*, 1972). Further, an increase in the degree of cytoplasmic oxidation in the glucose-treated unfed cows would imply a corresponding decrease in the [malate]/[oxaloacetate] ratio (Hohorst *et al.*, 1959), and consequently an increase in oxaloacetate concentration if the malate concentration did not decrease. An increased mitochondrial oxaloacetate concentration would be anti-ketogenic, since more oxaloacetate would be available for condensation with acetyl-CoA derived from fat oxidation, if citrate synthase (EC 4.1.3.7) were not rate-limiting. Previous work indicates that the activity of citrate synthase is not significantly decreased in unfed cows (Baird *et al.*, 1972).

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