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Gluconeogenesis in the Cow

THE EFFECTS OF A GLUCOCORTICOID ON HEPATIC INTERMEDIARY METABOLISM

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1. The hepatic concentrations of the ketone bodies and of metabolites and activities of enzymes involved in gluconeogenesis were measured in healthy lactating and non-lactating cows 48 h after administration of Voren, an ester of dexamethasone, and compared with those found in control animals given saline. Parallel measurements were also made of the blood concentrations of several of the metabolites. 2. Blood glucose concentrations were raised in the Voren-treated animals, whereas blood ketone body and free fatty acid concentrations were unaltered. Similarly there was no change in the hepatic concentrations of the ketone bodies. 3. Significant increases were found in the hepatic concentrations of citrate, 2-oxoglutarate and malate in both groups of animals given Voren. 4. The hepatic concentrations of those glycolytic intermediates that were measured either decreased or did not change after Voren treatment. 5. The enzymes aspartate transaminase and fructose 1,6-diphosphatase were unchanged in activity after Voren administration, whereas phosphopyruvate carboxylase (EC 4.1.1.32) activity was depressed in the lactating group. However, glucose 6-phosphatase, tryptophan oxygenase and tyrosine aminotransferase increased in activity. 6. In several cases those hepatic metabolites that increased in concentration after Voren administration were present in lower concentration in normal lactating cows than in normal non-lactating cows. The same applied *mutatis mutandis* to those metabolites that were decreased by Voren. 7. These findings are discussed in relation to the use of glucocorticoids in the treatment of bovine ketosis.

Recently accumulated evidence suggests that, in the ruminant liver, key enzymes involved in gluconeogenesis from the level of the citric acid cycle may be less responsive to physiological perturbations and to the external administration of glucocorticoids than is the case in the rat (Hibbitt & Baird, 1967; Baird *et al.* 1968; Ballard, Hanson, Kronfeld & Raggi, 1968; Filsell, Jarrett, Taylor & Keech, 1969; Heitzman, 1969). The major portion of the carbohydrate available to the ruminant is supplied by gluconeogenesis, and there must therefore be a continuous and rapid flux through this pathway even in the fed state (Annison & Lewis, 1959). For this reason, differences between ruminants and non-ruminants in the response of the gluconeogenic system to various stimuli might be expected.

Particular interest in the effect of glucocorticoids on gluconeogenesis in the cow arises from the fact that glucocorticoid administration is an effective therapy for the disease of bovine ketosis (Hatziolos & Shaw, 1950). In view of the complex interrelationship between the rates of fatty acid oxidation, ketogenesis and gluconeogenesis that has been demonstrated in the rat (e.g. Williamson, Browning & Olson, 1968), it is desirable that the antiketogenic action of glucocorticoids in the cow should be clarified.

In the present study, the hepatic concentrations of metabolites and activities of key enzymes involved in gluconeogenesis were measured in healthy lactating and non-lactating cows 48 h after administration of Voren (kindly supplied by Abbott Laboratories Ltd., Agro-Vet Division, Queenborough, Kent, U.K.), an ester of dexamethasone used for the treatment of bovine ketosis. These values were then compared with those found in control animals given saline. Especially noteworthy was the observation that glucocorticoid administration caused a significant increase in the hepatic concentrations of those citric acid-cycle intermediates that were measured, i.e. citrate, 2-oxoglutarate and malate. It is suggested that the ability of glucocorticoids to suppress ketosis in the

cow may be related to this increase in the quantity of citric acid-cycle components. Some of the findings have been reported in preliminary form (Baird & Heitzman, 1969*b*; Heitzman & Baird, 1969).

MATERIALS AND METHODS

Materials. Substrates and crystalline enzymes for enzyme assays and for metabolite determinations were obtained either from Boehringer Corp. (London) Ltd., London W.5, U.K., or from Sigma (London) Chemical Co. Ltd., London S.W.6., U.K. D(−)-3-Hydroxybutyrate dehydrogenase was prepared from *Rhodopseudomonas spheroides* cells by the method of Williamson, Mellanby & Krebs (1962), followed by further purification on DEAE-Sephadex A-50 (Pharmacia, Uppsala, Sweden) as recommended by Dr D. H. Williamson (personal communication). The cells were kindly provided by the Microbiological Research Establishment, Porton, Wilts., U.K. Other chemicals were of analytical grade. Double-distilled water, the second distillation being from glass, was used throughout.

Animals. Ayrshire and Friesian dairy cows were used. They were maintained under stall-fed conditions and received the standard diets given to cows in the dairy units at Compton. Lactating cows were subjected to experiment within the first 3 months of lactation, and had been through at least one previous lactation. Non-lactating cows were not in calf and had been through at least two lactations.

Glucocorticoid administration. Voren (dexamethasone 21-pyridine-4-carboxylate) was chosen for use in this work for the following reasons: (1) it is a highly active compound producing a satisfactory hyperglycaemic response; (2) Voren is used in the field for the treatment of bovine ketosis; (3) the ester form is considered to be pharmacologically active in the body for a greater length of time than is the free glucocorticoid.

Initial experiments showed that a single dose of Voren exerted a greater hyperglycaemic effect at 24h than at 48h after administration to healthy cows (Baird & Heitzman, 1969*a*). Nevertheless, 48h was chosen as the time that should be allowed to elapse after inoculation before examination of the effect of the glucocorticoid on hepatic metabolism. The choice of this period was based on three considerations: (1) the beneficial effects of glucocorticoid administration on bovine ketosis usually become apparent at about this time (Shaw, 1961); (2) long-term effects on the activity of key gluconeogenic and glycolytic enzymes should be demonstrable at 48h after inoculation; (3) it is well known that glucocorticoid administration temporarily depresses milk yield in cows (Meites, 1961), and initial investigations showed that the maximum decrease in milk yield (about 20%) occurred 48h after administration of 10mg of Voren to lactating cows (Baird & Heitzman, 1969*a*).

Baird & Heitzman (1969*a*) also observed that the hyperglycaemic response produced by a given quantity of Voren was greater in lactating than in non-lactating cows, and concluded that the hyperglycaemic response in the lactating animals might be potentiated by the concomitant fall in milk production. To ensure in the present study that observed changes in carbohydrate metabolism were in fact due to Voren administration and not merely to a partial inhibition of lactation, it was decided to study the effects of the glucocorticoid in both lactating cows and non-lactating cows.

The commercial preparation of Voren (1mg/ml) in iso-osmotic saline (0.9% NaCl) was injected intramuscularly. The lactating cows received 10mg, the dose recommended for the treatment of bovine ketosis, and the non-lactating cows 20mg. This in turn was the dose necessary to achieve an equivalent hyperglycaemia to that observed in lactating cows given 10mg of Voren (Baird & Heitzman, 1969*a*). The quantities of Voren injected were equivalent to an approximate dosage of 1mg/50kg body wt. in the lactating cows and of 1mg/25kg body wt. in the non-lactating cows. Administration of Voren at either dosage had no noticeable effect on appetite.

Liver tissue. At 48h after the administration of Voren or saline, liver tissue was obtained from the cows by using the biopsy method of Hibbitt & Baird (1967). The first sample of tissue removed from the caudate lobe was immediately freeze-clamped and subsequently extracted with $HClO_4$ as described by Baird *et al.* (1968). The neutralized extract was then used for the determination of steady-state concentrations of metabolites *in vivo*. Further liver samples were taken for enzyme assays and for the determination of glycogen.

Blood. Blood samples were taken immediately before liver biopsy. Two samples were taken. One was collected in 6% (w/v) $HClO_4$ as described by Baird *et al.* (1968) and the neutralized extract subsequently used for determination of glucose and blood metabolites. The other was collected together with heparin and the serum removed and used for determination of free fatty acid and amino acid content.

Hepatic enzyme activities. Phosphopyruvate carboxylase (EC 4.1.1.32) was determined essentially by the method of Seubert & Huth (1965). Fructose 1,6-diphosphatase (EC 3.1,3.11) was determined as described by Baird (1969), except that the concentration of Mg^{2+} in the assay medium was 5mM. Glucose 6-phosphatase (EC 3.1.3.9), tyrosine aminotransferase (EC 2.6.1.5), tryptophan oxygenase (EC 1.13.1.12), aspartate aminotransferase (EC 2.6.1.1) and pyruvate kinase (EC 2.7.1.40) were assayed as described by Heitzman (1969). All enzyme activities were expressed as μmol of substrate removed or product formed/min per g wet wt. at 37°C. Phosphopyruvate carboxylase and fructose 1,6 diphosphatase were assayed at 30°C and aspartate aminotransferase and pyruvate kinase at 25°C. In these cases activities at 37°C were calculated from the observed activities by assuming that the temperature coefficient (Q_{10}) for each enzyme was 2.

Metabolite concentrations in liver and blood. Metabolite concentrations were assayed in neutralized $HClO_4$-free extracts of blood or freeze-clamped liver. Lactate, pyruvate, 2-oxoglutarate, malate, triose phosphate, fructose 1,6-diphosphate, glycerophosphate, acetoacetate and D(−)-3-hydroxybutyrate were all determined as described by Baird *et al.* (1968). Citrate was measured by the method of Moellering & Gruber (1966) and glucose by the method of Pfleiderer (1963). Phosphoenolpyruvate, 2-phosphoglycerate and 3-phosphoglycerate were measured as described by Czok & Eckert (1963), together

with pyruvate, by the successive addition of lactate dehydrogenase, pyruvate kinase, enolase and phosphoglycerate mutase to the same cuvette.

Amino acids were assayed by using a Technicon Amino Acid Analyser, with a 21 h single-column system. The column was packed with Chromobead type A and eluted with a continuous gradient of 0.05M sodium citrate buffer, pH 2.88–5.00. The free amino acids contained in 1 ml of plasma or 1 ml of neutralized $HClO_4$-free liver extract were adsorbed on to 1 g of resin, which was then placed on the Analyser column for analysis as described by Reid (1966).

Further analyses. Glycogen was determined by the method of Pfleiderer (1963) in liver samples that had been stored frozen as soon as possible after biopsy. Free fatty acids were determined in fresh serum samples by using the test kit supplied by Dr Heinz Haury, Chemische Fabrik, Munich, Germany.

Statistics. The probability values (P) were obtained by Student's t test.

RESULTS

Blood constituents. The concentrations of glucose, ketone bodies and free fatty acids in the blood of cows 48 h after either Voren or saline administration and immediately before biopsy are compared in Table 1, which shows that blood glucose concentrations were significantly higher in Voren-treated animals at this stage. Ketone body concentrations and the hydroxybutyrate/acetoacetate ratio were essentially unaltered, however. Similarly, there was no significant difference in the concentrations of free fatty acids in the Voren-treated and saline-treated animals. Table 1 also shows that the concentration of 3-hydroxybutyrate was significantly higher in the lactating group than in the non-lactating group.

Table 2 records the blood concentrations of lactate, pyruvate, citrate and 2-oxoglutarate. Lactate and pyruvate concentrations in the Voren-treated animals were more variable than in the control animals. The only significant difference was the increase in pyruvate in the lactating group. The lactate/pyruvate ratio was unaltered. Voren administration caused slight but significant rises in blood oxoglutarate and citrate concentrations in the lactating and non-lactating groups respectively.

Liver metabolites. Table 3 compares the hepatic concentrations of various intermediates of the

Table 1. *Changes in the concentrations of glucose, free fatty acids and ketone bodies in the blood of lactating and non-lactating cows after Voren administration*

The concentration of glucose, 3-hydroxybutyrate and acetoacetate are expressed as μmol/ml of whole blood. The concentration of free fatty acids is expressed as μequiv./ml of plasma. The values are means ±S.D. with the numbers of observations in parentheses. The blood samples were taken 48 h after administration of either saline or Voren and immediately before liver biopsy. * $P<0.01$, † $P<0.001$ compared with saline controls; ‡ $P<0.01$ compared with lactating control.

Metabolite	Lactating cows + saline	Lactating cows + Voren	Non-lactating cows + saline	Non-lactating cows + Voren
Glucose	3.05 ±0.19 (6)	3.98 ±0.64* (5)	2.85 ±0.24 (6)	3.90 ±0.38† (6)
3-Hydroxybutyrate	0.54 ±0.14 (6)	0.70 ±0.32 (5)	0.25 ±0.11‡ (6)	0.23 ±0.07 (6)
Acetoacetate	0.030±0.021 (5)	0.040±0.024 (4)	0.011±0.004 (6)	0.011±0.005 (6)
3-Hydroxybutyrate/ acetoacetate ratio (mean)	18.0	17.5	22.7	20.9
Free fatty acids	0.28 ±0.07 (4)	0.38 ±0.15 (5)	0.28 ±0.08 (5)	0.23 ±0.09 (7)

Table 2. *Changes in the concentrations of various metabolites in the blood of lactating and non-lactating cows after Voren administration*

The concentrations of the metabolites are expressed as μmol/ml of whole blood. The values are means ±S.D. with the numbers of observations in parentheses. The blood samples were taken 48 h after administration of either saline or Voren and immediately before liver biopsy. * $P<0.05$ compared with saline controls; † $P<0.001$ compared with lactating control.

Metabolite	Lactating cows + saline	Lactating cows + Voren	Non-lactating cows + saline	Non-lactating cows + Voren
Lactate	0.94 ±0.64 (6)	1.69 ±1.45 (5)	0.82 ±0.30 (6)	1.19 ±0.50 (6)
Pyruvate	0.052±0.015 (6)	0.091±0.033* (5)	0.053±0.019 (6)	0.082±0.030 (6)
Lactate/pyruvate ratio (mean)	18.1	18.6	15.5	14.5
Citrate	0.16 ±0.01 (6)	0.14 ±0.05 (5)	0.10 ±0.01† (6)	0.12 ±0.02* (6)
2-Oxoglutarate	0.008±0.002 (6)	0.013±0.004* (5)	0.011±0.005 (6)	0.015±0.008 (6)

Table 3. *Changes in the concentrations of metabolites in the liver of lactating and non-lactating cows after Voren administration*

The concentrations of the metabolites are expressed as μmol/g wet wt. of tissue except for that of glycogen, which is expressed as μmol of glucose equivalent/g wet wt. of tissue. The values are means ±S.D. with the numbers of observations in parentheses. Where only two values were obtained both are given. The liver samples were obtained by biopsy 48h after administration of either saline or Voren. N.S., Not significant.

Metabolite	Lactating cows + saline (L)	Lactating cows + Voren (L+V)	Non-lactating cows + saline (NL)	Non-lactating cows + Voren (NL+V)	Significance of difference between means of respective groups of cows: L versus NL	L versus (L+V)	NL versus (NL+V)
Lactate	0.51 ±0.14 (6)	1.23 ±0.71 (6)	0.88 ±0.18 (5)	1.71 ±0.97 (6)	$P<0.01$	$P<0.05$	N.S.
Pyruvate	0.038±0.006 (6)	0.069±0.028 (6)	0.068±0.022 (5)	0.087±0.057 (6)	$P<0.02$	$P<0.05$	N.S.
Citrate	0.27 ±0.06 (5)	0.95 ±0.26 (6)	0.48 ±0.20 (5)	1.07 ±0.35 (6)	$P<0.05$	$P<0.001$	$P<0.01$
2-Oxoglutarate	0.099±0.044 (5)	0.378±0.153 (6)	0.284±0.094 (5)	0.489±0.180 (6)	$P<0.01$	$P<0.01$	$P<0.05$
Malate	0.43 ±0.07 (5)	1.32 ±0.67 (6)	0.52 ±0.18 (5)	1.44 ±0.34 (6)	N.S.	$P<0.02$	$P<0.001$
Phosphoenolpyruvate	0.122±0.025 (6)	0.107±0.050 (6)	0.073±0.034 (4)	0.065±0.025 (5)	$P<0.05$	N.S.	N.S.
2-Phosphoglycerate	0.046±0.015 (6)	0.047±0.021 (6)	0.042±0.027 (4)	0.042±0.005 (5)	N.S.	N.S.	N.S.
3-Phosphoglycerate	0.314±0.116 (6)	0.201±0.119 (6)	0.163±0.027 (4)	0.144±0.042 (5)	$P<0.05$	N.S.	N.S.
Glycerophosphate	0.38 ±0.14 (6)	0.29 ±0.04 (4)	0.25 ±0.13 (5)	0.20 ±0.07 (6)	N.S.	N.S.	N.S.
Triose phosphate	0.036, 0.045 (2)	0.051, 0.054 (2)	0.039±0.009 (3)	0.038±0.014 (4)	—	—	—
Fructose diphosphate	0.010, 0.061 (2)	0.005, 0.039 (2)	0.025±0.003 (3)	0.034±0.019 (4)	—	—	—
Glucose	7.92 ±2.45 (6)	6.78 ±1.30 (6)	6.22 ±2.58 (5)	6.23 ±1.35 (6)	N.S.	N.S.	N.S.
Glycogen	178 ±63 (4)	345 ±89 (5)	276 ±52 (6)	359 ±113 (6)	$P<0.05$	$P<0.02$	N.S.
3-Hydroxybutyrate	0.46 ±0.17 (6)	0.60 ±0.21 (6)	0.25 ±0.08 (5)	0.17 ±0.05 (6)	$P<0.05$	N.S.	N.S.
Acetoacetate	0.030±0.013 (5)	0.044±0.032 (5)	0.043±0.022 (5)	0.031±0.004 (6)	N.S.	N.S.	N.S.
3-Hydroxybutyrate/acetoacetate ratio (mean)	15.3	13.6	5.8	5.5	—	—	—
Lactate/pyruvate ratio	14.2 ±6.1 (6)	17.0 ±5.2 (6)	13.6 ±1.7 (5)	20.4 ±6.4 (6)	N.S.	N.S.	$P<0.05$

Embden–Meyerhof pathway and the citric acid cycle in cows 48h after the administration of Voren or saline. Similarity in the values for the lactate/pyruvate ratio in freeze-clamped liver and in blood collected in perchloric acid (Tables 2 and 3) in these experiments suggests that anaerobic changes had not occurred in the liver samples before freeze-clamping.

Results in Table 3 show that there were striking increases in the hepatic concentrations of those citric acid-cycle intermediates that were measured (i.e. citrate, 2-oxoglutarate and malate) as a result of Voren administration. The increases were statistically significant for each intermediate in both the lactating and non-lactating groups. In contrast, there were no significant changes in the concentrations of most of those intermediates of the Embden–Meyerhof pathway involved in gluconeogenesis from the citric acid cycle that were measured. Lactate and pyruvate were present in significantly greater concentrations in the Voren-treated animals in the lactating group, however, although this may have been a consequence of the apparent rise in the blood concentrations of these two compounds.

Free glucose concentrations in the liver did not change as a result of Voren administration. This was in contrast with the behaviour of glucose in the blood, but may have been related to the fact that glucose concentrations were in any case considerably higher in liver than in blood. Hepatic glycogen did increase in both groups, however. Ketone-body concentrations in the liver were similar to those in the blood and, as in the blood, no changes were observed in the hepatic concentrations of the ketone bodies after Voren administration. Neither was there any change in the ratio of the hepatic contents of hydroxybutyrate and acetoacetate.

Fig. 1 shows the percentage changes in the concentrations of selected metabolites 48h after Voren administration for both the lactating and the non-lactating groups of cows. Increases in the concentration of citric acid-cycle intermediates amounted to nearly 300% in some cases, whereas there were only relatively minor changes in the Embden–Meyerhof-pathway intermediates. The fact that malate increased by some 200% whereas phosphoenolpyruvate actually decreased in both groups of cows suggests the presence of a barrier to the unrestricted interconversion of these two intermediates via oxaloacetate and the malate dehydrogenase and phosphopyruvate carboxylase reactions.

Fig. 1 also clearly shows that the qualitative changes in metabolite concentration were very similar in the two groups of cows, although it is evident that the relative increases in the concentrations of citrate, oxoglutarate and glycogen were

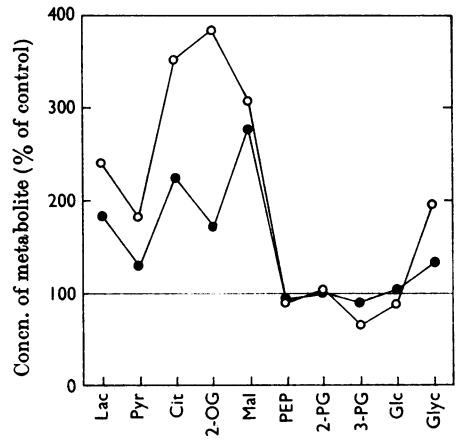

Fig. 1. Metabolites in cow livers after Voren administration. The values shown are the percentage changes in metabolite concentrations in the liver, compared with controls, when lactating cows (○) or non-lactating cows (●) were given Voren. The liver samples were obtained by biopsy 48h after administration of either saline or Voren. Key for metabolites: Lac, lactate; Pyr, pyruvate; Cit, citrate, 2-OG, 2-oxoglutarate; Mal, malate; PEP, phosphoenolpyruvate; 2-PG, 2-phosphoglycerate; 3-PG, 3-phosphoglycerate; Glc, glucose; Glyc, glycogen.

greater in the lactating group. Nevertheless the concentrations reached by these compounds after Voren treatment were essentially similar for both lactating and non-lactating cows. The difference in the proportional increases could be largely attributed to the lower hepatic concentrations of these three compounds in normal lactating cows than in normal non-lactating cows.

Amino acids in liver and blood. Table 4 lists the individual and total concentrations of the glucogenic amino acids aspartate, threonine, serine, glutamate, alanine and histidine in the liver, and the total concentrations of these six amino acids in blood plasma.

Administration of Voren brought about an apparent increase in the hepatic concentrations of glutamate and alanine in both groups of cows, although the only increase that was statistically significant was that for alanine in the lactating group. Voren had little or no effect on the hepatic concentrations of the other four amino acids. Although the change was not significant, the concentration of threonine appeared actually to decrease after Voren treatment. The reason for this may have been that on some occasions threonine and serine were not resolved in the eluate from the Amino Acid Analyser. There were slight increases in the total hepatic concentrations of the six amino acids in both groups of cows given Voren compared with the control groups. However, in neither case

Table 4. *Changes in the concentrations of glucogenic amino acids in liver and plasma of lactating and non-lactating cows after Voren administration*

The concentrations of the amino acids are expressed as μmol/g wet wt. of liver or μmol/ml of plasma. The values are means ±S.D. with the numbers of observations in parentheses. The total values are the means of the total of the six glucogenic amino acids obtained for each individual cow. The liver samples were obtained by biopsy 48h after administration of either saline or Voren, and the plasma samples immediately before each biopsy. *P* values compared with lactating cows given saline are: **P*<0.05; † *P*<0.01.

Amino acid	Lactating cows + saline (5)	Lactating cows + Voren (4)	Non-lactating cows + saline (4)	Non-lactating cows + Voren (4)
Liver				
Aspartate	1.05±0.53	0.96±0.60	0.44±0.13	0.54±0.10
Threonine	0.47±0.30	0.19±0.04	0.26±0.23	0.32±0.08
Serine	0.89±0.14	0.96±0.47	0.53±0.26*	0.54±0.12
Glutamate	3.34±1.19	4.01±1.80	2.96±1.49	3.86±0.48
Alanine	1.70±0.29	2.74±0.29†	1.18±0.11*	1.89±0.72
Histidine	0.31±0.09	0.38±0.04	0.47±0.09*	0.43±0.04
Total	7.17±1.45	9.02±3.03	5.29±2.17	7.58±0.45
Plasma				
Total	0.30±0.08 (4)	0.39±0.10 (4)	0.27±0.03 (4)	0.29±0.09 (4)

was the increase statistically significant. At 48h after Voren injection, the total concentrations of the six amino acids in blood plasma were very similar to the concentrations found in control samples (Table 4).

Liver enzyme activities. The effect of Voren administration on the activities of several key enzymes of gluconeogenesis and on pyruvate kinase, a key enzyme of glycolysis, are recorded in Table 5, which shows that the general trend in the response of the various enzymes to the glucocorticoid is similar in the lactating and the non-lactating groups. However, in several instances, there were differences in the statistical significance of the response. A second observation is that several of the gluconeogenic enzymes, which are reported to increase in activity in the rat after glucocorticoid administration (see Ashmore, Wagle & Uete, 1964), did not show any such increase in bovine liver under the experimental conditions of the present study. In particular, phosphopyruvate carboxylase and fructose 1,6-diphosphatase were not increased in activity 48h after Voren injection. In fact, phosphopyruvate carboxylase decreased in activity; the decrease in the lactating group was 28% and was statistically significant. Similarly, aspartate transaminase activity was not increased. There was, however, a moderate rise in the activity of glucose 6-phosphatase in response to Voren, with the maximum increase observed, i.e. that for the non-lactating group, amounting to about 38%. Tyrosine aminotransferase and tryptophan oxygenase, enzymes involved in the initial mobilization of amino acids, had also increased in activity 48h after Voren injection, although statistical significance was not found for tyrosine aminotransferase in the lactating group. The magnitude of the increases varied somewhat. Thus, although tyrosine aminotransferase increased by 84% in the non-lactating group, the increase in the lactating group was only 17%. Corresponding values for tryptophan oxygenase were 53% and 38%.

In summary, Table 5 shows that the capacity of the common gluconeogenic pathway from the citric acid cycle, as deduced from the measurement of the activities of key enzymes of gluconeogenesis *in vitro*, was not increased in bovine liver 48h after administration of either 10 or 20mg of Voren. A further point that emerges from Table 5 is that the gluconeogenic capacity of the liver of the lactating cow may be greater than that of the non-lactating cow, since the hepatic activity of phosphopyruvate carboxylase was some 33% lower in non-lactating than in lactating animals. However, this finding is in contrast with that of Ballard *et al.* (1968), who found no difference in the activities of the enzyme in lactating and non-lactating cows.

There appeared to have been a slight but significant decrease, amounting to 28%, in the activity of pyruvate kinase in the lactating group at 48h after Voren administration. This observation suggests that the response of key glycolytic enzymes to steroid administration may differ under some circumstances in the cow as compared with the rat. In the latter species, these enzymes are apparently unresponsive to steroids (Ashmore & Weber, 1968).

DISCUSSION

Liver metabolite concentrations and enzyme activities. This is the first report of the effect of glucocorticoid administration on the concentration of

Table 5. *Changes in the activities of enzymes related to gluconeogenesis in livers of lactating and non-lactating cows after Voren administration*

Activities are expressed as μmol of substrate consumed or product formed/min per g wet wt. of tissue at 37°C. The values are means ±S.D. with the numbers of observations in parentheses. The liver samples were obtained by biopsy 48h after administration of either saline or Voren. N.S., Not significant. The activity of phosphopyruvate carboxylase is the total activity present in the soluble and particulate fraction.

Enzyme	Lactating cows +saline (L)	Lactating cows +Voren (L+V)	Non-lactating cows +saline (NL)	Non-lactating cows +Voren (NL+V)	Significance of difference between means of respective groups of cows: L versus NL	L versus (L+V)	NL versus (NL+V)
Tyrosine aminotransferase	1.32 ±0.34 (6)	1.55 ±0.22 (5)	1.43 ±0.21 (7)	2.63 ±0.87 (6)	N.S.	N.S.	$P<0.01$
Tryptophan oxygenase	0.041±0.005 (7)	0.056±0.005 (6)	0.043±0.006 (7)	0.066±0.010 (6)	N.S.	$P<0.001$	$P<0.001$
Asparate aminotransferase	173.9 ±51.7 (8)	190.7 ±43.6 (6)	136.5 ±42.0 (6)	140.1 ±33.8 (5)	N.S.	N.S.	N.S.
Phosphopyruvate carboxylase (total)	12.35 ±2.30 (6)	8.90 ±2.23 (6)	8.33 ±2.20 (6)	6.52 ±1.33 (6)	$P<0.02$	$P<0.05$	N.S.
Fructose 1,6-diphosphatase	11.02 ±5.55 (6)	10.72 ±1.95 (5)	9.64 ±4.48 (5)	8.44 ±1.47 (5)	N.S.	N.S.	N.S.
Glucose 6-phosphatase	25.65 ±2.51 (11)	28.21 ±1.52 (8)	21.93 ±4.83 (7)	30.16 ±2.30 (6)	N.S.	$P<0.05$	$P<0.01$
Pyruvate kinase	15.34 ±3.21 (8)	10.98 ±0.93 (7)	12.40 ±2.22 (6)	10.46 ±1.89 (6)	N.S.	$P<0.01$	N.S.

metabolites of the citric acid cycle and the Embden–Meyerhof pathway in bovine liver. There have been a number of studies of the effects of glucocorticoids on hepatic metabolite concentrations in the rat, and these have not shown any marked alteration in the concentration of citric acid-cycle intermediates in the intact animal (Hornbrook, Burch & Lowry, 1965; Ray, 1968). The striking increases in the concentrations of components of the citric acid cycle in bovine liver seem therefore to represent a novel response to glucocorticoid treatment. However, in studies in the rat, attention has been directed to changes taking place within a relatively short space of time, and no information is available on metabolite concentrations at 48h after hormone administration.

The relative lack of responsiveness of key gluconeogenic enzymes to Voren treatment that has been observed in bovine liver in the present work is consistent with previous findings in ruminants (Hibbitt & Baird, 1967; Filsell *et al.* 1969). Particularly striking was the inability of Voren, at the concentrations used and under the experimental conditions, to cause any increase in the activity of fructose 1,6-diphosphatase, which has been widely reported to be sensitive to glucocorticoids in the rat (e.g. Ashmore & Weber, 1968). Nevertheless some of the bovine gluconeogenic enzymes did increase in activity after Voren administration. These were glucose 6-phosphatase, tyrosine aminotransferase and tryptophan oxygenase. In each case the response was greater in the non-lactating group given 20mg of Voren than in the lactating group given 10mg.

The decrease in phosphopyruvate carboxylase activity, statistically significant in the lactating group, is another effect of glucocorticoid administration that has not previously been reported in any species. The ability of glucocorticoids to decrease the activity of some key gluconeogenic enzymes may be another feature peculiar to ruminants, since Filsell *et al.* (1969) found that dexamethasone caused a 50% decrease in pyruvate carboxylase activity in sheep liver, although no effect was observed on phosphopyruvate carboxylase. These workers assayed phosphopyruvate carboxylase in the opposite direction to that used in the present study, however.

If in fact glucocorticoid administration causes an increased flow of gluconeogenic precursors to the liver in the cow as in the rat (Ashmore & Weber, 1968), the possibility arises that in the cow the rate of entry of carbon skeletons into the citric acid cycle under these circumstances is greater than the rate at which they are subsequently utilized for gluconeogenesis. This might then lead to an accumulation of material in the cycle of the type that has been observed in the present study.

Support for this possibility is given by the finding that Voren administration failed to increase the activity of phosphopyruvate carboxylase *in vitro*. This suggests that if the reverse Embden–Meyerhof pathway were already working at or near capacity in the normal animal, an increased flow of gluconeogenic precursors into the cycle could not be matched by a correspondingly increased rate of gluconeogenesis from the cycle via phosphoenolpyruvate. Further evidence that this may have been the case is provided by the fact that the phosphoenolpyruvate concentration failed to rise in conjunction with the rise in malate and citrate concentrations.

No values are available for hepatic glucose production in the cow. However, Katz & Bergman (1969) observed a maximum rate of glucose production by sheep liver of about 1.5 μmol/min per g, i.e. equivalent to the utilization of 3 μmol of oxaloacetate/min per g. If gluconeogenesis occurs at a similar rate in the cow, then it appears either that phosphopyruvate carboxylase is not rate-limiting in the normal animal and the gluconeogenic pathway is not working to capacity, or else that the effective activity of this enzyme *in vivo* is markedly lower than that determined *in vitro*. However, since phosphopyruvate carboxylase activity is evenly distributed between mitochondria and cytoplasm in bovine liver (Ballard *et al.* 1968), it is possible that the activity that should be taken into consideration as limiting the rate of gluconeogenesis may be restricted to only one of these two compartments. The relevant activity *in vitro* would then not be grossly different from that required for the rate of glucose production observed by Katz & Bergman (1969).

The fact that hepatic glycogen content increased suggests either that the overall rate of gluconeogenesis did rise somewhat, indicating that the full capacity of the pathway was not previously being utilized, or else that a rise in the concentration of insulin resulted in the activation of glycogen synthetase (EC 2.4.1.11) (Kreutner & Goldberg, 1967). There was some depression of the activity of pyruvate kinase, in the lactating group at least, and this should have diminished glycolysis in favour of gluconeogenesis (Krebs & Eggleston, 1965). Further, the rise in citrate concentration, possibly associated with a rise in that of ATP, might have had a similar effect as a result of the partial inhibition of phosphofructokinase (Sols, 1968; Brock, 1969).

Lactation and ketosis. In the present work, it was observed that the hepatic concentrations of citrate, 2-oxoglutarate, pyruvate, lactate and glycogen were lower in the control group of lactating cows than in the corresponding group of non-lactating cows, whereas the concentrations of phosphoenolpyruvate and 3-phosphoglycerate were higher. If one takes the non-lactating state as the norm, an effect of lactation is to cause a characteristic disturbance of carbohydrate metabolism with regard to the steady-state concentrations of metabolites. This is accompanied by a rise in the activity of phosphopyruvate carboxylase.

Further, the concentrations of hepatic intermediates that deviated from the norm as a result of lactation reverted towards normal values after Voren treatment, whereas phosphopyruvate carboxylase activity was depressed. In fact, the concentrations of citrate, oxoglutarate and glycogen increased well beyond the normal values. The con-

Table 6. *Comparison of metabolism in the livers of healthy lactating cows with that in ketotic cows, non-lactating cows and healthy lactating cows given Voren*

The results represent the percentage changes in the concentrations of the metabolites, or the activity of phosphopyruvate carboxylase, in ketotic cows, non-lactating cows, and lactating cows given Voren, compared with the corresponding values in normal lactating cows.

	Ketotic cows (% change)	Non-lactating cows‡ (% change)	Lactating cows‡ + Voren (% change)
Pyruvate	− 32*	+ 79	+ 82
Citrate	− 73†	+ 78	+252
2-Oxoglutarate	− 56*	+187	+282
Malate	+ 23*	+ 21	+207
Phosphoenolpyruvate	—	− 40	− 12
3-Phosphoglycerate	—	− 48	− 36
Glycogen	− 69*	+ 55	+ 94
Phosphopyruvate carboxylase	—	− 33	− 28

* Values computed from Baird *et al.* (1968) and Table 3.
† Value computed from Ballard *et al.* (1968) and Table 3.
‡ Values computed from Tables 3 and 5.

Table 7. *Calculated changes in hepatic oxaloacetate concentrations in lactating and non-lactating cows after Voren administration*

The changes in oxaloacetate concentration were calculated on the assumption that the lactate/pyruvate ratio and the malate/oxaloacetate ratio give the same value for the $NAD^+/NADH$ ratio in the cow as in the rat (Hohorst, Kreutz, Reim & Hübener, 1961), and that this should still apply after Voren administration.

	Lactate/pyruvate ratio	Malate (% change)	Oxaloacetate (% change calculated)
Lactating + saline	14.2	—	—
Lactating + Voren	17.0	+207	+156
Non-lactating + saline	13.6	—	—
Non-lactating + Voren	20.4	+177	+ 85

centration of malate, however, was similar in lactating and non-lactating cows, and yet was increased severalfold as the result of Voren treatment.

The work of Baird *et al.* (1968) and Ballard *et al.* (1968) demonstrated that the hepatic concentrations of oxoglutarate, citrate and glycogen were lower in the ketotic cow than in the healthy lactating cow. Baird *et al.* (1968) also found that pyruvate concentrations were lower in ketosis. It therefore seems that those metabolites that were at a depressed concentration in the healthy cow are at an even lower concentration in the ketotic cow. Evidently there is a range of possible concentrations of citric acid-cycle intermediates and glycogen, the actual concentrations depending on the physiological state of the animal. This situation is illustrated in Table 6. It can be deduced that one aspect of the therapeutic function of glucocorticoids in the treatment of bovine ketosis may be to restore the concentrations of intermediates of the citric acid cycle towards the values pertaining in the healthy lactating cow.

Oxaloacetate concentration plays a key role in determining the rate of ketone-body formation (Wieland & Löffler, 1963; Wieland, 1968) and hepatic concentrations of oxaloacetate were found to be depressed in bovine ketosis (Baird *et al.* 1968). Oxaloacetate was not assayed in the current study. However, if certain assumptions are made, the change in oxaloacetate concentration in healthy cows given Voren can be calculated (Table 7). On the basis of these calculations it seems that Voren may have substantially increased oxaloacetate concentrations in both groups of cows. A similar elevation of hepatic oxaloacetate concentration in ketotic cows could provide a rationale for the therapeutic action of glucocorticoid preparations.

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