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Effects of Starvation on Intermediary Metabolism in the Lactating Cow

A COMPARISON WITH METABOLIC CHANGES OCCURRING DURING BOVINE KETOSIS

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1. The purpose of this study was to determine the nature of the metabolic changes associated with carbohydrate and fat metabolism that occurred in the blood and liver of lactating dairy cows during starvation for 6 days. 2. During starvation, the blood concentrations of the free fatty acids and ketone bodies increased, whereas that of citrate decreased. After an initial increase, the blood concentration of glucose subsequently declined as starvation progressed. Starvation caused a significant decrease in the plasma concentration of serine and a significant increase in that of leucine. 3. After 6 days of starvation the hepatic concentrations of oxaloacetate, citrate, phosphoenolpyruvate, 2-phosphoglycerate, 3-phosphoglycerate, glucose, glycogen, ATP and NAD^+ had all decreased, as had the hepatic activities of phosphopyruvate carboxylase (EC 4.1.1.32) and pyruvate kinase (EC 2.7.1.40). 4. The above metabolic changes are similar to those previously found to occur in cows suffering from spontaneous ketosis (Baird *et al.*, 1968; Baird & Heitzman, 1971). 5. Milk yield decreased progressively during starvation. 6. There were marked differences in the ability of individual animals to resist the onset of severe starvation ketosis.

There is ample evidence that a negative energy balance is a prerequisite for the onset of bovine lactational ketosis. Further, the clinical condition is typically associated with inappetence (Hibbitt, 1967; Schultz, 1971). It therefore appeared to be of considerable importance to determine to what extent simple starvation can induce metabolic changes similar to those found to be associated with bovine ketosis (Bach & Hibbitt, 1959; Hibbitt & Baird, 1967; Baird *et al.*, 1968; Ballard *et al.*, 1968; Heitzman, 1969; Baird & Heitzman, 1971).

The number of previous studies concerned with total starvation in the lactating cow is limited. Robertson *et al.* (1960) monitored changes in the concentrations of certain blood metabolites during a 6-day starvation period, and Ballard *et al.* (1968) observed metabolic changes in blood and liver in cows that had been starved for 4 days. These two studies were in agreement in finding that starvation induces a rise in the concentrations of free fatty acids and ketone bodies in the blood. Robertson *et al.* (1960) also observed a sharp fall in citrate concentration in the blood, and Ballard *et al.* (1968) observed a fall in citrate concentration and rises in ketone-body concentrations in the liver. Two of the cows starved by Robertson *et al.* (1960) developed milk fever (hypocalcaemia).

In the work described in the present paper, five lactating dairy cows were each starved for 6 days. The effects of this 6-day starvation on hepatic intermediary metabolism are described in detail, and further information is provided on changes occurring in blood metabolite concentrations during the starvation period. The results are assessed in the light of previous work on starvation and on bovine ketosis.

Experimental

Materials

Substrates and enzymes for metabolite determinations and enzyme assays 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. Other chemicals were of analytical grade. Double-distilled water, both distillations being from glass, was used throughout. [1-^{14}C]Acetyl-CoA was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Animals

Five lactating Friesian × Ayrshire dairy cows from the Institute's herd were used in this study. At the time of starvation the period elapsed since calving varied between 25 and 59 days. All the animals had been through at least one previous lactation. Before starvation the animals had been fed on a standard dairy concentrate ration, containing 15.3% protein, 58.5% nitrogen-free extract and 3.5% fibre, together with medium-quality hay and high-dry-matter silage. The values obtained for hepatic

metabolite and enzyme concentrations in the starved cows are compared in the text with the values previously obtained for normal lactating cows (Heitzman, 1969; Baird & Heitzman, 1970, 1971). The starved cows were from the same herd as these normal animals and were maintained under identical stall-fed conditions before starvation.

Methods

Starvation procedure. The cows were starved of all food one at a time for 6 days (i.e. 144h) in a loose-box fitted with a wooden slatted floor. In one corner of the box a thick rubber mat was laid across the slats for the animal to lie on. Water was supplied *ad lib.* The animals were milked twice daily in the loose-box with a bucket unit, and the milk yields were recorded.

Blood. Blood was collected from the jugular vein of each cow immediately before starvation began and before the animal was moved into the loose-box. Subsequent samples of jugular-vein blood were then collected daily during the starvation period, when extreme care was taken not to excite the animal before or during the bleeding. The blood samples were analysed as described by Baird & Heitzman (1970). The samples taken at the end of the starvation period were obtained immediately before liver biopsy (see below).

Liver tissue. At the end of the starvation period (i.e. 144h after commencing starvation) liver tissue was obtained by biopsy and used for metabolite and enzyme assays as described by Baird & Heitzman (1970). To avoid unnecessarily exciting the animal, the hair was clipped from the sub-lumbar fossa before starvation began and the cow remained in the loose-box for the surgery, which was carried out under a local anaesthetic containing no adrenaline (Xylocaine 2%, supplied by Astra Chemicals Ltd., Watford, U.K.).

Similar biopsies were performed on a further four cows that were in early lactation and that had not been starved. Liver tissue from these animals was used to obtained control values for the activity of phosphopyruvate carboxylase (EC 4.1.1.32).

Steady-state metabolite concentrations in liver and blood. The methods of assay of the following compounds were those used by Baird & Heitzman (1970): lactate, pyruvate, D(−)-3-hydroxybutyrate, acetoacetate, citrate, malate, 2-oxoglutarate, phosphoenolpyruvate, 2-phosphoglycerate, 3-phosphoglycerate, α-glycerophosphate, glucose and glycogen. The hepatic concentrations of oxaloacetate, NAD^+, NADH, ATP, ADP and AMP were assayed as described by Baird & Heitzman (1971). Plasma amino acid concentrations were determined with an amino acid analyser, as described by Baird & Heitzman (1970).

Hepatic enzyme activities. The activities of phosphopyruvate carboxylase (EC 4.1.1.32), glucose 6-phosphatase (EC 3.1.3.9) and pyruvate kinase (EC 2.7.1.40) were assayed as described by Baird & Heitzman (1970). Citrate synthase (EC 4.1.3.7) was assayed by the method of Srere *et al.* (1963).

Free fatty acids. Free fatty acids were determined in plasma samples with the test kit supplied by Boehringer Corp. (London) Ltd. The method used in this kit is based on that of Duncombe (1964).

Statistics. The probability values (P) were obtained by Student's t test. Daily observations of blood metabolite concentrations and milk yield during starvation were analysed by analysis of variance.

Results

Blood metabolites

The effects of starvation on the blood concentrations of various compounds involved in carbohydrate and fat metabolism are recorded in Table 1. From the middle of the 6-day starvation period onwards the blood concentrations of free fatty acids and ketone bodies were greatly increased in the five cows, whereas those of citrate, glucose and pyruvate were decreased, as compared with the normal values. These metabolic changes are strongly reminiscent of those observed in cows suffering from spontaneous ketosis (Baird *et al.*, 1968; Baird & Heitzman, 1971).

Changes in the concentrations of several of the metabolites had already taken place after 24h of starvation. The most marked of these was the increase of 635% in the free fatty acid concentration. There was also a transient, but significant, increase in the concentration of glucose, of 32%. The third compound to be affected was citrate, which had decreased slightly, but not significantly, in concentration. In spite of the elevation of the free fatty acid concentration there was no increase in the concentrations of the ketone bodies at this time.

After 48h of starvation the concentrations of the free fatty acids had risen still further, to 960% of the initial value, and the concentrations of the ketone bodies had now also begun to rise. In conjunction with these changes the concentration of citrate had fallen to only 21% of the normal value (cf. Robertson *et al.*, 1960).

Over the remainder of the starvation period there were further rises in the concentrations of both free fatty acids and ketone bodies to 1300 and 960%, and falls in the concentrations of glucose and pyruvate to 64 and 66%, of the normal values respectively. The concentration of citrate remained at the low plateau value reached after 48h. A consequence of these alterations in metabolite concentrations was that the [lactate]/[pyruvate] ratio rose by 45% and the [hydroxybutyrate]/[acetoacetate] ratio fell by 64% as starvation progressed.

Table 1. *Daily blood metabolite concentrations in starved 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 means for the five animals and were examined by analysis of variance. Mean values for a given parameter differing significantly from that at day 0 are indicated by *. There were large changes in the concentrations of hydroxybutyrate, acetoacetate, citrate and free fatty acids over the starvation period taken as a whole. For these compounds, therefore, analyses of variance were done on the logarithms of individual values, and standard errors were calculated for the means for days 2–6 only, over which period concentrations were more uniform.

	Concentration or ratio							
Time of starvation (days) ...	0	1	2	3	4	5	6	S.E.M.
Lactate	0.55	0.55	0.53	0.74	0.63	0.63	0.55	0.099
Pyruvate	0.050	0.058	0.031*	0.043	0.036*	0.039	0.033*	0.004
[Lactate]/[pyruvate]	11.9	9.9	17.2	17.3	17.1	15.7	17.3	2.1
3-Hydroxybutyrate	0.42	0.42	0.87*	1.98*	2.80*	3.43*	2.86*	0.45
Acetoacetate	0.04	0.06	0.24*	0.43*	0.66*	0.98*	0.80*	0.103
[3-Hydroxybutyrate]/[acetoacetate]	9.7	7.7	4.2*	4.7*	4.2*	3.5*	3.5*	0.9
Citrate	0.138	0.107	0.029*	0.019*	0.020*	0.021*	0.025*	0.004
Glucose	2.72	3.59*	2.28	2.04	2.10	2.03	1.73*	0.24
Free fatty acids	0.17	1.25*	1.63*	2.05*	2.19*	2.23*	2.14*	0.17

By contrast with these findings, Robertson *et al.* (1960) observed a rise in the concentrations of both glucose and pyruvate during prolonged starvation. These authors did not use enzymic methods of metabolite estimation, however.

Milk yield

Starvation caused a steady decline in milk yield. As Table 2 shows, this parameter had decreased on average by 25% after only 24h of starvation and by 44% after 48h. At the end of the starvation period milk yield was only 25% of that immediately before starvation. Similar observations were made by Robertson *et al.* (1960).

Table 2. *Daily milk yields in starved cows*

Milk yield is expressed as kg of milk produced during the preceding 24h. The cows were milked twice during each 24h period. The values are means for the five animals and were examined by analysis of variance. The mean value for day 0 differed significantly from the mean values on all the subsequent days of starvation.

Time of starvation (days)	Milk yield (kg)
0	17.6
1	13.2
2	9.9
3	7.2
4	5.6
5	5.0
6	4.3

Hepatic metabolites

Table 3 lists the steady-state concentrations of various intermediates of the tricarboxylic acid cycle and the Embden–Meyerhof pathway in the livers of the cows at the end of the 6-day starvation period. The differences between these concentrations and those found previously in normal fed animals (Baird & Heitzman, 1970, 1971) are also recorded, as are the significances of these differences.

Starvation caused significant decreases in the concentrations of citrate, oxaloacetate, phosphoenolpyruvate, 2-phosphoglycerate, 3-phosphoglycerate, glucose and glycogen. These changes, taken as a whole, presumably reflect a decreased availability of gluconeogenic precursors during starvation. As Table 3 also shows, they were accompanied by much-increased concentrations of both hydroxybutyrate and acetoacetate, and a decrease in the [hydroxybutyrate]/[acetoacetate] ratio. The [lactate]/[pyruvate] ratio increased during starvation (Table 3).

The mean hepatic concentrations of the adenine nucleotides and of NAD^+ for all five starved cows are recorded in Table 4, as is the concentration of NADH for one of the cows. Starvation apparently caused a slight but significant fall in the concentration of ATP, without affecting the concentrations of either ADP or AMP. Further, the concentration of NAD^+ was decreased to half that found in the fed state, and the one value for NADH suggested that

this compound had increased in concentration relative to the normal value (Baird & Heitzman, 1971). Taken together, the values for NAD^+ and NADH indicate that the [total NAD^+]/[total NADH] ratio may have fallen significantly as a result of starvation.

Amino acids in blood plasma

Table 5 lists the concentrations of amino acids in blood plasma samples obtained from the five cows at the beginning and at the end of the starvation period. Starvation caused decreases in the concentrations of most of the glucogenic amino acids, although the only decrease that was statistically significant was that for serine. Starvation also led to increases in the concentration of a number of the other amino acids, notably those of the group consisting of leucine, valine and isoleucine. Of these increases, only that for the ketogenic leucine was statistically significant, however.

Hepatic enzymes. The activities in the livers from

Table 3. *Hepatic metabolite concentrations in lactating cows starved for 6 days*

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. The control values were those found in healthy cows in early lactation. That for oxaloacetate was from Baird & Heitzman (1971), and the remainder were from Baird & Heitzman (1970). The significance of differences between mean values for starved and control animals was determined by Student's *t* test. Abbreviation: N.S., not significant.

	Concentration		
Metabolite	(μmol/g wet wt.)	(% of control value)	Significance
Lactate	1.02 ±0.57 (5)	200	N.S.
Pyruvate	0.024±0.016 (5)	63	N.S.
Citrate	0.075±0.027 (5)	28	$P<0.001$
2-Oxoglutarate	0.083±0.075 (5)	84	N.S.
Malate	0.75 ±0.45 (5)	174	N.S.
Oxaloacetate	$2.54\times10^{-3}\pm0.67\times10^{-3}$ (4)	52	$P<0.02$
Phosphoenolpyruvate	0.042±0.039 (5)	34	$P<0.01$
2-Phosphoglycerate	0.018±0.012 (5)	39	$P<0.01$
3-Phosphoglycerate	0.099±0.097 (5)	32	$P<0.01$
Glycerophosphate	0.48 ±0.15 (5)	126	N.S.
Glucose	3.23 ±1.31 (5)	41	$P<0.01$
Glycogen	32.8 ±41.0 (5)	18	$P<0.01$
3-Hydroxybutyrate	3.00 ±1.98 (5)	652	$P<0.02$
Acetoacetate	0.65 ±0.50 (5)	2167	$P<0.05$
[3-Hydroxybutyrate]/[acetoacetate] (mean)	4.6	30	—
[Lactate]/[pyruvate] (mean)	42.5	300	—

Table 4. *Hepatic adenine nucleotide and nicotinamide–adenine dinucleotide concentrations in lactating cows starved for 6 days*

Concentrations are expressed as μmol/g wet wt. of tissue. The values are means ± S.D., with the numbers of observations in parentheses. The control values were those found in healthy cows in early lactation (Baird & Heitzman, 1971). Significance of results was determined as described in Table 3.

	Concentration		
Metabolite	(μmol/g wet wt.)	(% of control value)	Significance
ATP	0.88±0.13 (5)	73	$P<0.05$
ADP	1.08±0.17 (5)	85	N.S.
AMP	0.67±0.11 (5)	100	N.S.
NAD^+	0.39±0.10 (5)	48	$P<0.001$
(NADH	0.21 (1)	159	—)

Table 5. *Plasma amino acid concentrations in lactating cows immediately before, and 6 days after, commencement of starvation*

The concentration of the amino acids are expressed as μmol/ml of plasma. The values are means for the five cows and were examined by analysis of variance. *$P<0.05$; **$P<0.01$, for significance of difference between values before and after starvation.

	Concentration			
Amino acid	Before starvation	At completion of 6 days of starvation	S.E.M.	Change (%)
Asp	0.034	0.022	0.007	−35
Thr	0.086	0.048	0.027	−44
Ser	0.081	0.027**	0.009	−67
Glu	0.105	0.047	0.034	−55
Gly	0.360	0.262	0.089	−27
Ala	0.171	0.107	0.043	−37
Val	0.178	0.275	0.062	+54
Ile	0.099	0.122	0.025	+23
Leu	0.103	0.246*	0.065	+139
Tyr	0.030	0.025	0.053	−17
Phe	0.032	0.039*	0.004	+22
Lys	0.058	0.077	0.016	+33
His	0.040	0.055	0.014	+38
Arg	0.051	0.029	0.018	−43

Table 6. *Hepatic enzyme activities in lactating cows starved for 6 days*

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 control values were those found in healthy cows in early lactation. Those for pyruvate kinase and glucose 6-phosphatase were from Heitzman (1969). That for phosphopyruvate carboxylase was reassayed in the present work and was 23.4±4.3 for four cows, and that for citrate synthase was 2.61±0.51 for seven cows. Significance of results was determined as described in Table 3.

	Activity		
Enzyme	(μmol/min per g wet wt.)	(% of control value)	Significance
Phosphopyruvate carboxylase	16.6±2.6 (4)	71	$P<0.05$
Pyruvate kinase	5.0±0.8 (5)	33	$P<0.001$
Glucose 6-phosphatase	28.0±4.3 (5)	109	N.S.
Citrate synthase	2.13±0.07 (4)	82	N.S.

the starved cows of phosphopyruvate carboxylase, pyruvate kinase, glucose 6-phosphatase and citrate synthase were assayed *in vitro* (Table 6). The first three of these enzymes were assayed in previous studies because of their potential importance in regulating gluconeogenesis (Baird & Heitzman, 1970, 1971). The activity of phosphopyruvate carboxylase was significantly decreased as a result of starvation. Butler & Elliot (1970) also found a decrease in the activity of phosphopyruvate carboxylase in partially starved cows, but the decrease was not statistically significant. The activity of pyruvate kinase was also depressed in the current study, in this case highly significantly, whereas that of glucose 6-phosphatase was unaltered. Finally, Table 6 shows that the activity of citrate synthase appeared to be decreased slightly as a result of starvation, although this change was not statistically significant.

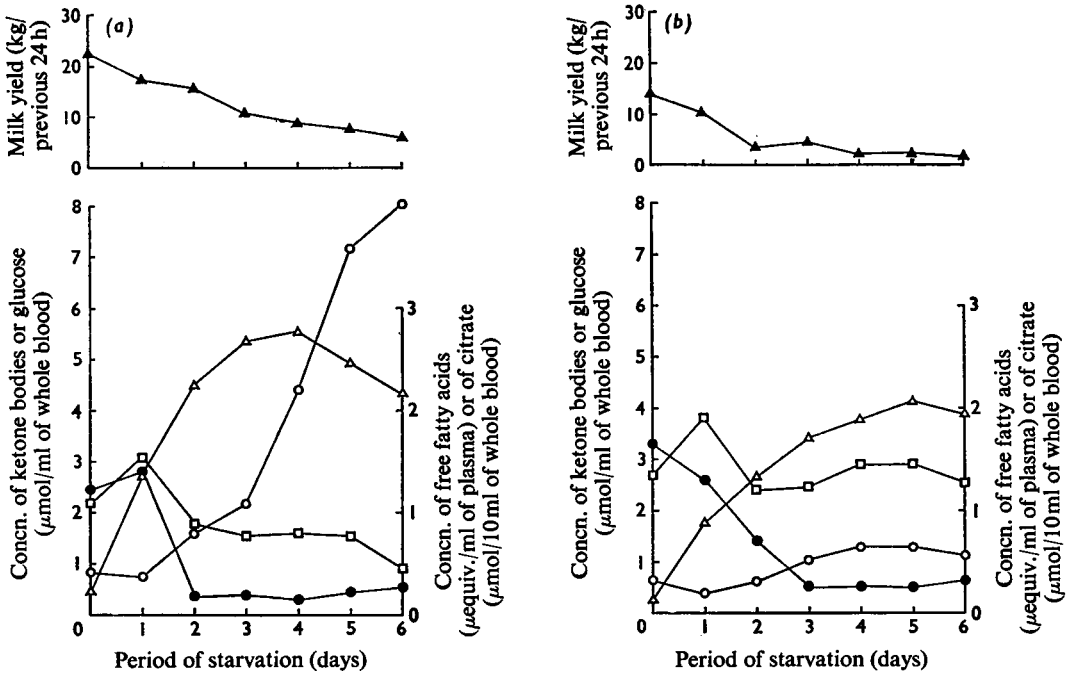

Fig. 1. *Daily concentrations of ketone bodies* (○), *free fatty acids* (△), *glucose* (□) *and citrate* (●) *and the milk yield* (▲) *in two cows during the* 6 *day starvation period*

(*a*) Values for cow A, in which severe hyperketonaemia and hypoglycaemia developed during starvation. (*b*) Values for cow B, in which there was only mild hyperketonaemia and no hypoglycaemia during starvation.

Variation in susceptibility to starvation ketosis

The results discussed above refer to mean values for the five cows. Examination of the metabolic changes in the individual animals revealed, however, that there was a considerable variation in the ability of the cows to resist the development of severe hyperketonaemia, even though other metabolic changes appeared to be common to all the animals. This variation suggested that there might be two types of metabolic change, i.e. that associated with starvation *per se* and that associated with the severe ketosis arising as a result of prolonged starvation.

Two of the cows represented extreme cases in this respect. The one animal (cow A), which had calved 25 days previously, became severely hyperketonaemic and hypoglycaemic by the end of the 6-day period, whereas the other (cow B), which had calved 59 days previously, exhibited only mild hyperketonaemia and normoglycaemia. The changes in the concentrations of the various blood metabolites with duration of starvation are plotted for the two cows in Figs. 1(*a*) and 1(*b*) respectively. Fig. 1 shows that, apart from the differences in ketone-body and glucose concentrations, the two animals both exhibited marked rises in free fatty acid concentrations and falls in citrate concentrations during the starvation period. The rise in free fatty acid concentration in cow B was smaller than that in cow A but was, even so, greater than that normally found in spontaneously ketotic cows (see the Discussion section). The decrease in citrate concentration was of a similar magnitude in both animals, however.

The daily milk yields of the two cows are also plotted in Fig. 1. These graphs show that the milk yield of cow B was only 67% of that of cow A before starvation. The yields of both animals decreased as starvation progressed. However, the rate of this decrease was greater for cow B. Thus after 2 days of starvation the milk yield of cow B was only 25% of the pre-starvation value, whereas that of cow A was still 71%. Because of these differences, cow A produced substantially more milk during the starvation period than did cow B: whereas cow A produced 66.5kg, cow B produced only 25kg.

Comparison of metabolite concentrations in the livers of cow A and cow B indicated that the metabolic changes at the level of the tricarboxylic acid cycle and the Embden–Meyerhof pathway were very

similar in the two cases, i.e. depressed concentrations of citrate and of phosphoenolpyruvate, and 2- and 3-phosphoglyceric acid, were found in both animals. However, the hepatic concentrations of glycogen and glucose were considerably higher in cow B than cow A, whereas those of the ketone bodies were considerably lower.

Discussion

Starvation ketosis and spontaneous ketosis

Table 1 shows that lactating cows subject to prolonged starvation become ketotic, when ketosis is defined as a large accumulation of ketone bodies in the blood. In fact the average ketone-body concentrations reached during starvation were similar to those observed previously in spontaneously ketotic cows (Baird *et al.*, 1968; Baird & Heitzman, 1971). The other changes in blood and liver metabolite concentrations occurring during starvation in this current work were also similar to those found in clinical cases of spontaneous ketosis. The question arises, therefore, whether there are in fact any differences in intermediary metabolism between cows suffering from starvation ketosis and cows suffering from spontaneous ketosis.

As far as the present work is concerned it is clear that no absolute differences were found in the type of metabolic changes occurring in starvation ketosis and spontaneous ketosis. There were, however, differences of degree in a number of cases. With regard to the blood metabolite concentrations, an obvious difference, confirming previous observations (e.g. Kronfeld, 1971), was that the concentration of free fatty acids in the blood of the five starved cows was higher than that observed in spontaneous ketosis. Thus after 5 days of starvation the concentration was 2.22 ± 0.45 μequiv./ml, whereas that in four cows suffering from spontaneous ketosis was 1.02 ± 0.34 μequiv./ml ($P < 0.01$) (G. D. Baird, unpublished work). In fact, the mean value for the cows starved for only 1 day was higher than this mean for spontaneously ketotic cows, and yet was not associated with any rise in ketone-body concentrations above the normal value. A second difference for blood metabolites was in the magnitude of the decrease in the citrate concentration. In the five starved cows the blood citrate concentration was 0.021 ± 0.004 μmol/ml on day 5 of starvation, whereas in spontaneously ketotic cows it was 0.045 ± 0.023 μmol/ml ($P < 0.05$) (Baird & Heitzman, 1971).

There were no statistically significant differences between the metabolite concentrations in the livers of the starved cows (Tables 3 and 4) and those in spontaneously ketotic cows (Baird *et al.*, 1968; Baird & Heitzman, 1971).

Of the hepatic enzyme activities, a decrease in the activity of phosphopyruvate carboxylase was also observed in spontaneous ketosis, although the change was not statistically significant in this case (Baird & Heitzman, 1971). Pyruvate kinase activity was also decreased in spontaneous ketosis, but a difference here appears to be that the extent of the decrease was less, i.e. the activity was decreased by 67% in starvation but only by 34% in ketosis ($P < 0.01$, for difference between values for starved and ketotic animals) (Baird & Heitzman, 1971). A further possible enzymic difference noted by Ballard *et al.* (1968) was that pyruvate carboxylase activity was elevated in starvation but not in spontaneous ketosis (see also Filsell *et al.*, 1969). However, observations from this laboratory provide evidence in favour of the reverse situation (G. D. Baird & J. L. Young, unpublished work).

The similarity of the metabolic changes occurring in starvation ketosis and spontaneous ketosis suggest that they have a common origin in the two instances. The nature of the changes indicate that they are due primarily to a shortage of carbohydrate precursors and that a consequence of this shortage may be a decreased rate of gluconeogenesis. This in turn may lead to an imbalance between the demand for glucose and the rate at which glucose can be supplied. Previous studies with sheep have demonstrated that glucose entry rate is decreased in starvation (see Lindsay, 1970) and that the rate of glucose synthesis by the liver is decreased during starvation ketosis (Katz & Bergman, 1969).

Chronological sequence of events in starvation

A noteworthy point emerging from the present work is that 24h of starvation is sufficient to elicit an extensive metabolic reaction in the cow, even though there is still presumably a considerable quantity of utilizable food left in the rumen (see Blaxter, 1967). The fact that the blood concentrations of both glucose and free fatty acids rose within this period of time, and that the rise in glucose concentration was only transient, suggest that these changes may have been due to the release of a hormone, possibly noradrenaline, in response to the stimulus of inanition (see Bergman, 1971). Another possibility is that the changes were occasioned by the concomitant decrease in milk yield of 25%.

The metabolic changes that occurred during subsequent days of starvation were presumably imposed on the animal as a direct result of the prolonged absence of any exogenous supply of gluconeogenic precursors. The steep fall in blood citrate concentration on day 2 may have been associated with a similar decrease in the liver, indicating that the changes in the concentrations of hepatic metabolites that were observed after 6 days of starvation may in fact have taken place up to 4 days earlier. Such changes on day

2 would help to explain the observed rise in blood ketone-body concentrations at this time.

Variation in susceptibility to starvation ketosis

It is probable that the difference in the resistance of the two cows A and B (Figs. 1*a* and 1*b*) to the development of starvation ketosis is related to the time elapsed since calving. Thus it is known that the susceptibility of dairy cows to spontaneous ketosis decreases with the length of lactation beyond the sixth week *post partum* (see, e.g., Schultz, 1971). This decrease in susceptibility is usually considered to be related to the decline in milk yield, which begins at about this time and which indicates that milk production is no longer taking the same degree of precedence in the utilization of the animal's resources. This loss of precedence may also explain the superior ability of cow B to switch off milk production in response to starvation (Fig. 1).

These arguments assume that the severity of the ketosis developing during starvation is directly related to the demands made by milk production on carbohydrate resources. An alternative explanation of the relationship between the maintenance of milk production and severe starvation ketosis would be that the higher rate of milk production is due to a greater availability of 3-hydroxybutyrate, which is a precursor for milk-fat synthesis (Linzell *et al.*, 1967). However, Kronfeld *et al.* (1968) found that the rate of uptake of hydroxybutyrate by the mammary gland was not significantly higher in lactating cows starved for 4 days than in normal lactating cows. Availability of hydroxybutyrate would not, therefore, seem to be a factor in determining the level of milk production during starvation.

Comparison of starvation-induced metabolic changes in the cow and the rat

In general the changes in hepatic metabolite concentrations in starved cows are similar to those previously observed in starved rats (see Williamson *et al.*, 1967; Start & Newsholme, 1968; Wieland, 1968; Williamson *et al.*, 1969; Greenbaum *et al.*, 1971). One striking difference, however, is that while there is unanimous agreement that the citrate concentration in both liver and blood falls markedly in cows during starvation (Robertson *et al.*, 1960; Ballard *et al.*, 1968; the present paper), there are conflicting views on whether the concentration of citrate changes during starvation in the rat (Start & Newsholme, 1968; Wieland, 1968; Greenbaum *et al.*, 1971).

The behaviour of the two gluconeogenic enzymes phosphopyruvate carboxylase and glucose 6-phosphatase is clearly very different in the starved cow from that observed in the starved rat, in which species activity is increased as a result of starvation (see Pontremoli & Grazi, 1968).

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