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Effects of Food Deprivation on Ketonaemia, Ketogenesis and Hepatic Intermediary Metabolism in the Non-Lactating Dairy Cow

By G. DAVID BAIRD, RAYMOND J. HEITZMAN, IAN M. REID, HERBERT W. SYMONDS and MICHAEL A. LOMAX*
Agricultural Research Council, Institute for Research on Animal Diseases, Compton, Newbury, Berks. RG16 0NN, U.K.

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1. The aim of this work was to investigate why non-lactating dairy cows are less susceptible to the development of ketonaemia during food deprivation than are dairy cows in early lactation. 2. The first experiment (Expt. A) consisted of determining the effect of 6 days of food deprivation on the concentrations of ketone bodies, and of metabolites related to the regulation of ketogenesis, in jugular blood and liver of non-lactating cows. 3. During the food deprivation, blood ketone-body concentrations rose significantly, but to a value that was only 16% of that achieved in lactating cows deprived of food for 6 days [Baird, Heitzman & Hibbitt (1972) *Biochem. J.* **128**, 1311–1318]. 4. In the liver, food deprivation caused: a rise in ketone-body concentrations; a fall in the concentration of glycogen and of various intermediates of the Embden–Meyerhof pathway and the tricarboxylic acid cycle; an increase in cytoplasmic reduction; a decrease in the [total NAD^+]/[total NADH] ratio; a decrease in energy charge. These changes were all qualitatively similar to those previously observed in the livers of the food-deprived lactating cows. 5. There appeared therefore to be a discrepancy in the food-deprived non-lactating cows between the absence of marked ketonaemia and the occurrence of metabolic changes within the liver suggesting increased hepatic ketogenesis. This discrepancy was partially resolved in Expt. B by the observation in two catheterized non-lactating cows that, although there was a 2-fold increase in hepatic ketogenesis during 6 days of food deprivation, ketogenesis from the splanchnic bed as a whole (i.e. gut and liver combined) declined slightly owing to cessation of gut ketogenesis.

Robertson *et al.* (1960) and Baird *et al.* (1972) demonstrated that mature cows that are in the early stages of lactation, i.e. about 25–50 days *post partum*, develop severe ketonaemia when deprived of food for 4–6 days. In more advanced lactation susceptibility to the induction of food-deprivation ketosis appeared to decline. These findings complement observations made by several authors that the susceptibility of lactating cows to spontaneous ketosis is greatest between 20 and 35 days *post partum* and that uncomplicated spontaneous ketosis is rarely observed later than 75 days *post partum* (e.g. Halse & Mogstad, 1975).

It seems therefore that the intensity of lactation may be a crucial factor in determining the susceptibility of non-pregnant cows to ketosis, since the lactational peak has been passed at 60–70 days *post partum*. It follows that non-lactating non-pregnant cows are likely to be particularly resistant to the induction of marked ketonaemia by means of food deprivation for short periods.

*Present address: Biological Laboratory, University of Kent, Canterbury, Kent CT2 7NJ, U.K.

Baird *et al.* (1972) found that the induction of ketosis in lactating cows by means of food deprivation was associated with major changes in the hepatic steady-state concentrations of a variety of metabolites, including intermediates of the tricarboxylic acid cycle and the Embden–Meyerhof pathway, ATP and NAD^+. These changes were very similar to those previously found to be associated with spontaneous ketosis (Baird & Heitzman, 1971), and it was concluded that the changes in metabolite concentration, particularly the fall in the concentrations of glycogen and of intermediates of the tricarboxylic acid cycle, were a necessary prerequisite for elevated hepatic ketogenesis.

To obtain further information on the relationship between hepatic metabolite concentrations and ketonaemia, the effect of food deprivation has now been investigated in non-lactating, non-pregnant cows. It was reasoned that the absence of any major increase in blood ketone-body concentrations in the food-deprived non-lactating cows might indicate an absence of any significant increase in hepatic ketogenesis. If this were the case, little change might then

be expected in the concentrations of relevant hepatic metabolites if change in metabolite concentration and increased hepatic ketogenesis were directly related.

It was in fact found that after non-lactating cows were deprived of food for 6 days, ketone-body concentrations in jugular blood had increased significantly, but only to values typically found in healthy, fed, lactating cows. However, in spite of the absence of any marked ketonaemia, food deprivation of the non-lactating cows had produced changes in the concentrations of hepatic metabolites that were qualitatively similar to those seen previously in the food-deprived lactating cows of Baird *et al.* (1972) that became severely ketotic. This was investigated further by monitoring ketone-body production by the liver and gut *in vivo* in appropriately catheterized animals. This showed that hepatic ketogenesis doubled in the non-lactating cows after 4–6 days of food deprivation. However, gut production of ketone bodies ceased, so that net production of ketone bodies from the splanchnic bed (i.e. gut plus liver) showed a moderate decline.

Experimental

Materials

Substrates and enzymes for determination of metabolite concentrations were obtained from Boehringer Corp. (London) Ltd., Lewes, Sussex, U.K. *p*-Aminohippuric acid, used for the determination of blood flow rate, was obtained from Sigma (London) Chemical Co., Kingston upon Thames, Surrey, U.K. Other chemicals were of analytical grade.

A total of 11 non-pregnant, non-lactating Friesian × Ayrshire dairy cows were used. All the cows had had at least two lactations, and in most cases the last calving had taken place at least 1 year before the animal was used for experiment. While being fed, each animal received a daily maintenance ration of approx. 5kg of medium-quality hay and 2kg of dairy concentrate. The latter contained 16% (w/w) crude protein. The daily feed was given in two equal portions, the first being given at about 07:30h and second at about 14:00h.

Methods

Experimental procedure. Two separate experiments were carried out (Expts. A and B). Expt. A was designed to determine the effects of 6 days of food deprivation on steady-state metabolite concentrations in the liver and blood. Expt. B was designed to determine the effect of this period of food deprivation on the production rates *in vivo* of ketone bodies, butyrate and long-chain non-esterified fatty acids, across the liver and the gut (for ketone bodies) or the liver alone (for butyrate and non-esterified fatty acids).

In Expt. A, nine of the cows were divided randomly into two groups containing five and four animals respectively. In the former group, which was the food-deprived group, each of the cows in turn was deprived of all food, but not water, for 6 days (144h). The procedure was as follows. On the day on which food deprivation commenced, i.e. day 0, the cow was given the morning feed, and a blood sample (see below) was taken at about 09:30h (zero time). No further food was given for the next 144h, but further blood samples were taken at intervals of 24h, counting from zero time. At 144h after zero time the final blood sample was taken and the animal was immediately subjected to liver biopsy (see below). The cow was not fed until the biopsy was completed. Apart from the fact that no milking was involved, the food-deprivation procedure was otherwise identical with that described for lactating cows by Baird *et al.* (1972).

In the latter of the two groups, i.e. the fed group, the cows were fed normally. In each case, a blood sample was obtained at about 09:30h on the day of experiment and then the cow was immediately subjected to liver biopsy.

In Expt. B, two cows were used that had been permanently catheterized (see below). The data in this experiment represent the mean metabolite production rates (see below) that were observed in these cows on 3 separate days in the fed state, on the one hand, and on days 4 and 6 of food deprivation on the other. Averaging the values for days 4 and 6 of food deprivation appeared justified, since little difference was observed in production rates on these days. The data for Expt. B were in fact taken from a larger study involving two cows in mid-lactation as well (G. D. Baird, H. W. Symonds, I. M. Reid, C. J. Roberts & M. A. Lomax, unpublished work). The procedure for each cow in this larger study was as follows. The animal was first allowed to recover after surgery, which took some 2–3 weeks. Subsequently, while the cow was feeding normally, metabolite production rates were measured on 3 separate days between 11:00 and 13:00h on each occasion. The last of these days was taken as day 0 of the food-deprivation period and the procedure for food deprivation was as described above. Metabolite production rates were then re-measured on days 1, 2, 4 and 6 of food deprivation, and then again after 1 and 3 days of re-feeding. The statistical analysis of the data in Expt. B is based on the analysis of this larger study.

Liver tissue. This was obtained by biopsy via a laparotomy. The piece of liver was freeze-clamped immediately after removal and subsequently extracted with 30% (w/v) $HClO_4$. The extract, neutralized with 20% (w/v) KOH, was then used for the determination of the steady-state concentrations of hepatic metabolites. The overall procedure was as described by Baird *et al.* (1968), except that, before

the laparotomy incision was made, local anaesthesia of the sub-lumbar fossa was achieved with an adrenaline-free anaesthetic (2% xylocaine: Astra Chemicals, Watford, Herts, U.K.).

Blood. Blood was obtained from the jugular vein by venepuncture in Expt. A, and from various blood vessels via catheters in Expt. B, and at each bleeding separate samples were collected in 6% (w/v) $HClO_4$, and in heparin, for metabolite assays (Baird & Heitzman, 1970).

Measurement of production rates in vivo. This was carried out on the two cows in which catheters had been permanently implanted by surgical techniques into the portal vein at the porta hepatis, into an hepatic vein, a carotid artery and a mesenteric vein. The procedure for catheterization was a combination of the methods of Symonds & Baird (1973) and Baird *et al.* (1975), except that silicone-rubber cannulae [silastic tubing (Dow Corning): Lepetit Pharmaceuticals, Hounslow, Middx. U.K.] were used in place of the polyvinyl cannulae previously used. The procedure for measuring net production rates across the liver and gut was essentially as described by Baird *et al.* (1975). *p*-Aminohippuric acid was used to measure blood flow rate, and net metabolite production rates were then calculated from the values for blood flow rate and from the observed metabolite concentrations in blood derived from the portal vein, the hepatic vein and the carotid artery (see Katz & Bergman, 1969*a*).

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 and glycogen. Glucose, dihydroxyacetone 1,6-phosphate, glyceraldehyde phosphate, fructose bisphosphate and fatty non-estrified acids, in Expt. A, were assayed by the methods used by Treacher *et al.* (1976). ATP, ADP, AMP, NAD^+ and NADH were assayed by the methods used by Baird & Heitzman (1971). Amino acid concentrations were determined with an amino acid analyser (Bio-Cal BC200; LKB Instruments, South Croydon, U.K.). The concentration of non-esterified fatty acids in the blood of cannulated cows in Expt. B was determined by using t.l.c. The method was as follows. Total lipid was extracted into chloroform from a known volume of plasma by shaking the plasma with methanol and chloroform, essentially as described by Atkinson *et al.* (1972). A portion of the chloroform extract was evaporated to dryness and the lipid content weighed accurately. A portion of the remainder of the extract was subjected to t.l.c. to separate the various lipid classes into discrete spots according to the method of Nelson (1972). The percentage of total lipid in each spot was then determined spectrophotometrically after charring with conc. H_2SO_4 (Kritchevsky *et al.*, 1973). The concentration of non-esterified fatty acid was finally calculated from the percentage of total lipid represented by the appropriate spot and from the weight of total lipid per unit volume of plasma. The mean mol.wt. of the fatty acids was taken to be 256 (the mol.wt. of hexadecanoic acid).

All the hepatic metabolite concentrations were determined in the neutralized, perchloric acid extracts of freeze-clamped liver. All the concentrations of blood metabolites were determined in neutralized perchloric acid extracts of blood, except concentration of the non-esterified fatty acids, which was determined in heparinized plasma.

Butyrate was determined directly in acidified perchloric acid extracts of blood by g.l.c., with a series 204 gas chromatograph (Pye–Unicam, Cambridge, U.K.). The column was that used by Baird *et al.* (1975).

Statistics. Expt. A. The significance of differences between metabolite concentrations in different groups of cows was determined by Student's *t* test. Daily observations of blood metabolite concentrations during food deprivation in the non-lactating cows were analysed by analysis of variance.

Expt. B. In the larger experiment, of which Expt. B was a part, the data were adjusted for the fed value for each cow and were analysed by a split-plot analysis of variance with lactation group as a main-plot effect and day of sampling as a sub-plot effect.

Results and Discussion

Metabolite concentrations in jugular blood

As Table 1 shows, exposure of non-lactating cows to 6 days of food deprivation in Expt. A did in fact lead to a highly significant increase in ketone-body concentrations in jugular blood. However, the concentration reached by the end of the food-deprivation period was only 3 times that before food deprivation and was still of the same order as that seen in healthy, fed cows in early lactation (see, e.g., Baird & Heitzman, 1970). By contrast, food deprivation caused the concentration of non-esterified fatty acids to increase some 6–7-fold and to reach a concentration higher than that found in lactating cows with spontaneous ketosis (Baird *et al.*, 1972). The food deprivation also elicited a highly significant decrease in the blood concentration of citrate and in the blood [3-hydroxybutyrate]/[acetoacetate] ratio. In general, food deprivation had little effect on the blood concentrations of lactate, pyruvate and glucose.

Some of the above changes in metabolite concentration in the jugular blood of the non-lactating cows are compared in Figs. 1(*a*) and 1(*b*) with corresponding changes previously observed in the food-deprived cows in early lactation (Baird *et al.*, 1972). As Fig. 1(*a*) shows, during food deprivation the

Table 1. *Effect of food deprivation on daily metabolite concentrations in non-lactating cows (Expt. A)*
Concentrations are expressed as mM in whole blood, except for that for non-esterified fatty acids, which is expressed as μequiv./ml of plasma. The values are means for five animals and were examined by analysis of variance. Significances of differences between means on day 0 and those measured on subsequent days of food deprivation are: * < 0.05; ** < 0.01; *** < 0.001. S.E.M., Standard error of difference between means.

	Concentration or ratio on given day of food deprivation							
Metabolite Day ...	0	1	2	3	4	5	6	S.E.M.
3-Hydroxybutyrate	0.17	0.25	0.34***	0.42***	0.47***	0.51***	0.50***	0.05
Acetoacetate	0.009	0.012	0.024	0.040*	0.054***	0.093***	0.067***	0.011
Lactate	0.71	0.52	0.64	0.67	0.71	0.62	0.60	0.09
Pyruvate	0.035	0.035	0.039	0.038	0.038	0.035	0.033	0.005
Citrate	0.078	0.060*	0.037***	0.034***	0.032***	0.031***	0.027***	0.007
Glucose	2.61	2.60	2.55	2.47	2.45	2.62	2.35*	0.10
Non-esterified fatty acids	0.33	0.79*	1.56***	1.73***	1.83***	2.47***	1.68***	0.19
[3-Hydroxybutyrate]/ [acetoacetate]	23.3	22.1	14.7**	11.1***	10.2***	6.1***	7.7***	2.9
[Lactate]/[pyruvate]	21.0	14.8	16.6	16.6	19.2	18.1	19.3	3.3

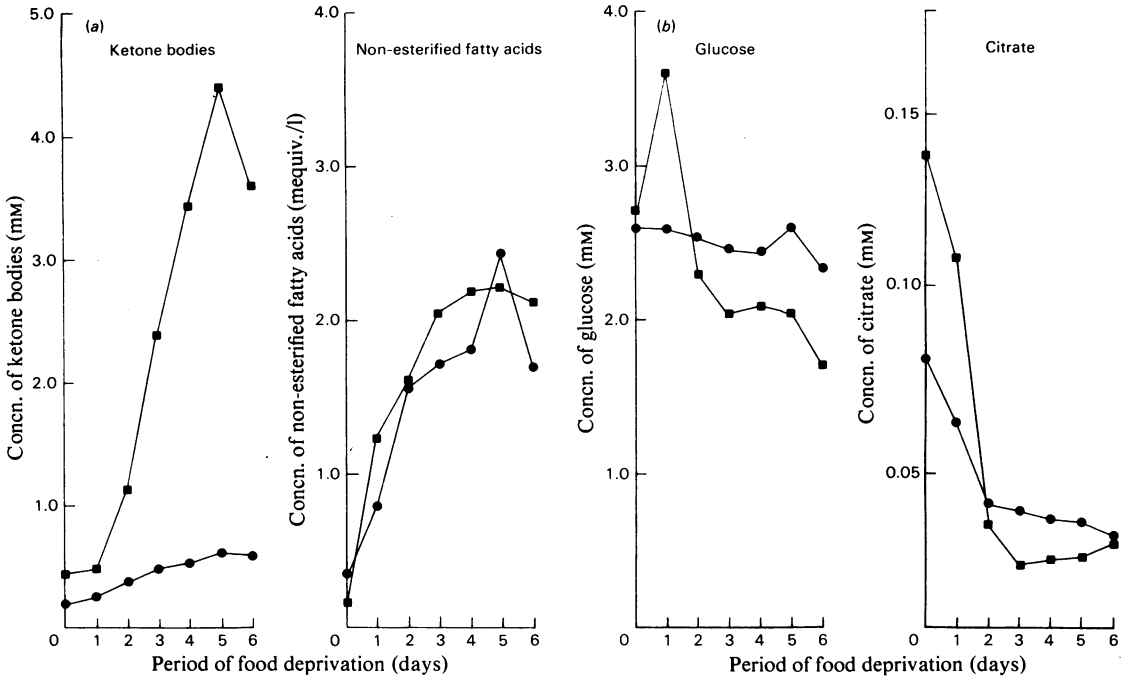

Fig. 1. *Daily blood concentrations of ketone bodies, non-esterified fatty acids, glucose and citrate in non-lactating cows (●) and in lactating cows (■) during 6 days of food deprivation*
(*a*) Concentrations of ketone bodies and non-esterified fatty acids and (*b*) concentrations of glucose and citrate. The values plotted are means for all the cows in each respective group. Those for the lactating cows are taken from Baird *et al.* (1972).

ketone-body concentrations in the lactating cows rose to values that were similar to those found in cows with spontaneous ketosis (see, e.g., Baird & Heitzman, 1971). However, the rise in ketone-body concentrations was much less in the non-lactating cows, the values reached being only some 14% of those reached in the lactating cows. Despite this, the concentrations of non-esterified fatty acids reached

during food deprivation were similar in both the lactating and non-lactating cows and, as mentioned above, higher even than those seen in spontaneously ketotic cows.

Fig. 1(*b*) shows that food deprivation caused greater proportional changes in the blood concentration of glucose and citrate in the lactating cows than the non-lactating cows, although the citrate concentration fell to similar values in both groups.

There is some indication in Table 1 that the concentrations of acetoacetate, non-esterified fatty acids and glucose were all lower on day 6 of food deprivation than on day 5. Similar decreases in concentration were observed between days 5 and 6 of food deprivation in the lactating cows as well (Baird *et al.*, 1972). A possible explanation for this phenomenon is that the endogenous supply of readily mobilizable glucogenic precursors and non-esterified fatty acids was becoming exhausted at this time in both groups of cows.

Further experiments (not tabulated) showed that the 6 days of food deprivation had relatively little effect on blood amino acid content in the non-lactating cows. Any numerical changes in concentration that did occur were generally of a smaller magnitude than the changes in amino acid concentration that were previously observed in lactating cows subjected to food deprivation (Baird *et al.*, 1972), and not necessarily in the same direction. Nevertheless, food deprivation did induce a significant increase in blood leucine concentration in the non-lactating cows, as it

Table 2. *Comparison of hepatic metabolite concentrations in fed non-lactating cows and in non-lactating cows deprived of food for 6 days (Expt.)*

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 significances of differences between mean values were determined by Student's *t* test. The values for metabolite concentrations in food-deprived lactating cows, with which the values for the food-deprived non-lactating cows are compared, were obtained from Baird *et al.* (1972). Abbreviation: N.S., not significant ($P \geqslant 0.05$).

	Concentration or ratio		Comparison of values for food-deprived non-lactating cows with: (A) Fed non-lactating cows (FN)		(B) Food-deprived lactating cows (F-DL)	
Metabolite	Fed non-lactating cows	Food-deprived non-lactating cows	% of value for FN	Significance of difference between means	% of value for F-DL	Significance of difference between means
3-Hydroxybutyrate	0.37 ±0.27 (4)	1.03 ±0.13 (5)	276	< 0.01	34	N.S.
Acetoacetate	0.035±0.004 (3)	0.084±0.010 (3)	240	< 0.001	13	N.S.
Lactate	0.75 ±0.29 (4)	1.51 ±0.73 (5)	202	N.S.	148	N.S.
Pyruvate	0.074±0.024 (4)	0.057±0.044 (5)	77	N.S.	238	N.S.
Citrate	0.38 ±0.20 (4)	0.18 ±0.08 (5)	49	N.S.	245	< 0.02
2-Oxoglutarate	0.26 ±0.06 (4)	0.046±0.014 (5)	18	< 0.001	55	N.S.
Malate	0.23 ±0.10 (4)	0.75 ±0.19 (5)	334	< 0.01	100	N.S.
Phosphoenolpyruvate	0.088±0.022 (4)	0.053±0.014 (5)	60	< 0.05	126	N.S.
2-Phosphoglycerate	0.041±0.006 (4)	0.024±0.006 (5)	59	< 0.01	133	N.S.
3-Phosphoglycerate	0.26 ±0.07 (4)	0.18 ±0.06 (5)	69	N.S.	177	N.S.
Glycerol 1-phosphate	0.18 ±0.13 (3)	0.79 ±0.17 (5)	432	< 0.01	165	< 0.02
Dihydroxyacetone phosphate	0.034±0.008 (4)	0.061±0.024 (5)	179	N.S.	—	—
Glyceraldehyde phosphate	0.016±0.004 (4)	0.012±0.004 (5)	75	N.S.	—	—
Fructose bisphosphate	0.034±0.015 (4)	0.043±0.025 (5)	126	N.S.	—	—
Glucose	4.91 ±1.87 (4)	7.82 ±3.23 (5)	159	N.S.	242	< 0.02
Glycogen	269.5±96.1 (4)	77.6 ±11.9 (4)	29	< 0.01	237	N.S.
[3-Hydroxybutyrate]/[acetoacetate]	13.0 ±10.3 (3)	13.0 ±2.6 (3)	100	N.S.	283	—
[Lactate]/[pyruvate]	10.3 ±3.5 (4)	38.7 ±21.7 (5)	376	< 0.05	91	—
[Glycerol 1-phosphate]/[dihydroxyacetone phosphate]	5.1 ±1.9 (3)	14.7 ±6.1 (5)	288	< 0.05	—	—

had previously been found to do in the lactating animals. However, this increase amounted to only 24% compared with 139% in the lactating cows.

It is evident therefore that exposure to 6 days of food deprivation had substantially less effect on the jugular blood concentrations of the ketone bodies and of glucose and amino acids in the non-lactating cows in the present work than in the lactating cows that were studied by Baird *et al.*, (1972). In these respects, therefore, the intermediary metabolism of the non-lactating cow is subject to less distortion during food deprivation than is that of the cow in early lactation. That food deprivation was having major effects on some blood metabolite concentrations was, however, indicated by the fact that the fall in citrate concentration and rise in non-esterified fatty acid concentration were as great as had previously been observed in the lactating cows. Of interest is the finding that the concentrations of non-esterified fatty acids and citrate had already changed significantly after only 24h of food deprivation.

Jarrett *et al.* (1976) found that the total arterial concentration of ketone bodies increased from 0.3 to 1.2mM in wethers that were deprived of food for 6 days. This might indicate that sheep were more susceptible than cows to the development of food-deprivation ketonaemia. Evidence to the contrary is, however, provided by Herriman & Heitzman (1978), who found that after 6 days of food deprivation the jugular blood concentration of total ketone bodies was still only 0.43mM in non-pregnant, non-lactating ewes. The non-lactating cow appears to be much more resistant to the induction of hyperketonaemia and hypoglycaemia by food deprivation than is the male rat. Thus, 48h of food deprivation is sufficient to cause the circulating concentration of the ketone bodies in the male rat to rise from 0.1 to 2.1mM and that of glucose to fall from 6.0 to 4.3mM (Williamson *et al.*, 1969). Sex differences are unlikely to be involved here since the available evidence indicates that females are more susceptible than males to ketonaemia induced by food deprivation (e.g. Deuel & Gulick, 1932; MacKay *et al.*, 1941).

Hepatic metabolite concentrations

Table 2 lists the steady-state metabolite concentrations that were found in the livers of the fed and food-deprived groups of non-lactating cows. It is evident that the 6 days of food deprivation caused a trebling of the concentration of ketone bodies in the liver, as it did in the blood (cf. Table 1). However, the ketone-body concentration was 2–3 times as great in the liver as in jugular blood in both the fed and food-deprived states (i.e. liver concentrations of 0.41 and 1.11mM compared with blood concentrations of 0.18 and 0.57mM respectively; cf. Tables 1 and 2), the difference between liver and blood concentrations during food deprivation being highly significant ($P < 0.001$). Disparity between liver and blood concentrations of ketone bodies was not observed in the food-deprived lactating cows studied by Baird *et al.* (1972). Consequently, the difference between the ketone-body concentrations in the food-deprived lactating and non-lactating cows was less in the liver than in the blood [cf. Fig. 1(*a*) and Table 2]. In cows with spontaneous ketosis, ketone-body concentrations are somewhat higher in blood than in liver (see, e.g. Baird *et al.*, 1968; Baird & Heitzman, 1971).

As Table 2 also shows, depriving the non-lactating cows of food led to decreases in the hepatic concentrations of glycogen, citrate, 2-oxoglutarate, phosphoenolpyruvate, 2-phosphoglycerate and 3-phosphoglycerate. Of these decreases, those for glycogen, 2-oxoglutarate, phosphoenolpyruvate and 2-phosphoglycerate were statistically significant. Food deprivation also caused increases in the hepatic concentrations of lactate, malate, glycerol 1-phosphate, dihydroxyacetone phosphate and glucose. In this case, the increases for malate and glycerol 1-phosphate were statistically significant. The values for the [lactate]/[pyruvate] and [glycerol 1-phosphate]/[dihydroxyacetone phosphate] ratios given in Table 2 indicate that the hepatic cytoplasm was more reduced in the food-deprived state than in the fed state. The values for these ratios correspond in turn to values for the cytoplasmic [free NAD^+]/[free NADH] ratio of 1832 and 2203 respectively in the fed state, and 488 and 764 respectively in the food-deprived state. In making the calculations, the mass-action equilibrium constants for the lactate dehydrogenase (EC 1.1.1.27) and glycerol 1-phosphate dehydrogenase (EC 1.1.1.8) reactions are taken to be 0.53×10^{-4} and 0.89×10^{-4} respectively at pH7.0 (Hohorst *et al.*, 1959).

The 6 days of food deprivation also induced a 65% fall, which was statistically highly significant, in the hepatic concentration of ATP in the non-lactating cows (Table 3). As a result of the fall in ATP concentration, the hepatic energy charge (Atkinson, 1968) was 32% lower in the non-lactating cows during food deprivation than in those in the fed state. Table 3 also shows that the hepatic [total NAD^+]/[total NADH] ratio fell in the non-lactating cows during food deprivation by 51%, entirely as the result of a fall in the concentration of NAD^+. It will be noticed that in the food-deprived animals the total recovery of adenine nucleotides was 30% (i.e. 1 μmol/g wet wt. of liver) less than in those in the fed state, whereas recovery of NAD was 33% (i.e. 0.24 μmol/g wet wt.) less. Similar decreases in the recovery of the adenine nucleotides and of NAD were previously observed in the lactating cows during food deprivation, and amounted to 16% (i.e. 0.51 μmol/g wet wt.) and 36% (i.e. 0.34 μmol/g wet wt.) respectively (Baird & Heitzman, 1971; Baird *et al.*, 1972).

All the changes in metabolite concentration,

Table 3. *Comparison of hepatic adenine nucleotide and NAD concentrations in fed non-lactating cows and in non-lactating cows deprived of food for 6 days (Expt. A)*

The concentrations of the metabolites are expressed as μmol/g wet wt. of tissue. The values are means ± S.D. with the numbers of observations in parentheses. The significances of differences between mean values were determined by Student's *t* test. The values for metabolite concentrations in food-deprived lactating cows, with which the values for the food-deprived non-lactating cows are compared, were obtained from Baird *et al.* (1972). Abbreviation: N.S., not significant ($P \geqslant 0.05$).

	Concentration or ratio		Comparison of values for food-deprived non-lactating cows with: (A) Fed non-lactating cows (FN)		(B) Food-deprived lactating cows (F-DL)	
Metabolite	Fed non-lactating cows	Food-deprived non-lactating cows	% of value for FN	Significance of difference between means	% of value for F-DL	Significance of difference between means
ATP	1.71 ± 0.24 (4)	0.59 ± 0.20 (5)	35	< 0.001	67	< 0.05
ADP	1.08 ± 0.06 (4)	0.98 ± 0.17 (5)	91	N.S.	91	N.S.
AMP	0.66 ± 0.12 (4)	0.86 ± 0.18 (5)	130	N.S.	128	N.S.
[ATP] + ½[ADP]/([ATP] + [ADP] + [AMP])	0.65 ± 0.03 (4)	0.44 ± 0.04 (5)	68	< 0.001	—	—
NAD^+	0.60 ± 0.08 (4)	0.35 ± 0.05 (5)	58	< 0.001	90	N.S.
NADH	0.13 ± 0.04 (3)	0.14 ± 0.03 (3)	108	N.S.	—	—
$[NAD^+]/[NADH]$	4.7 ± 0.8 (3)	2.3 ± 0.3 (3)	49	< 0.01	—	—

Table 4. *Effect of food deprivation on production rates of ketone bodies across the liver and gut, and of non-esterified fatty acids and butyrate across the liver alone, in the two catheterized non-lactating cows in vivo (Expt. B)*

Production rates, i.e. rates of net exchange, are expressed as mmol/min in whole blood, except for that for non-esterified fatty acids, which is expressed as mmol/min in plasma. Positive values indicate output and negative values indicate uptake. The values for the fed state are the means of six observations and those for the food-deprived state are the means of four observations (see the Experimental section): $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ (compared with the fed state).

Metabolite	Organ	Fed	Food-deprived	S.E.M.
D-3-Hydroxybutyrate	Liver	2.94	3.92	0.69
	Gut	2.28	−0.02**	0.53
	Liver + gut	5.22	3.90	0.76
Acetoacetate	Liver	−0.79	0.41**	0.32
	Gut	0.64	−0.05**	0.16
	Liver + gut	−0.15	0.36	0.23
Total ketone bodies	Liver	2.15	4.33*	0.92
	Gut	2.94	−0.05**	0.65
	Liver + gut	5.07	4.31	0.92
Non-esterified fatty acids†	Liver	−0.31	−1.23	0.86
Butyrate	Liver	−1.05	0***	0.17

† From I. M. Reid, R. A. Collins, G. D. Baird, C. J. Roberts & H. W. Symonds (unpublished work).

redox state and energy charge that took place in the livers of the non-lactating cows during food deprivation (Tables 2 and 3) are qualitatively similar to those that were earlier observed to occur in the food-deprived lactating cows, which became severely hyperketonaemic. Furthermore, the changes were of the type that in earlier studies from this laboratory have been considered to be associated with an increase in hepatic ketogenesis (Baird *et al.*, 1968; Baird & Heitzman, 1971; Baird *et al.*, 1972). This suggested, therefore, that the resistance of the non-lactating cows to the development of hyperketonaemia during food deprivation could not be attributed to the absence of any increase in hepatic ketogenesis. In order to gain some insight into this apparent paradox it seemed necessary to determine how changes that

occurred in hepatic ketogenesis during food deprivation related to changes in the output of ketone bodies from the splanchnic bed as a whole, bearing in mind that, in the ruminant, ketone bodies are also produced from the epithelium of the rumen wall during feeding (Katz & Bergman, 1969*b*; Baird *et al.*, 1975). This point was examined in Expt. B.

Production rates in vivo

Table 4 records the rates of production of ketone bodies *in vivo* that were observed across the liver and gut separately, and across the splanchnic bed as a whole (i.e. gut and liver combined), in the two catheterized non-lactating cows in the fed state, and then after food deprivation, in Expt. B. Table 4 also records the rate of production of non-esterified fatty acids and butyrate across the liver alone on the same occasions. In the fed state there was a net output of hydroxybutyrate by the liver, but a net uptake of acetoacetate. Hydroxybutyrate output was greater than acetoacetate uptake, however, so that there was a net output of ketone bodies of about 2mmol/min. The effect of food deprivation was to cause a moderate increase in hepatic output of hydroxybutyrate and at the same time to transform the hepatic uptake of acetoacetate, characteristic of the fed state, into an output of some 0.4mmol/min. As a consequence of these changes, net hepatic output of ketone bodies during food deprivation was twice as great as in the fed state (Table 4). However, in spite of this increase in hepatic ketone-body output, there was actually a (non-significant) decline in ketone-body output from the splanchnic bed as a whole during food deprivation, as Table 4 shows. This slight decline in output was due to the fact that gut output of ketone bodies ceased during food deprivation, and the contribution of some 3mmol/min that the gut made to net output in the fed state was consequently lost. It is also evident from Table 4 that butyrate, if quantitatively converted, could account for half of the liver output of ketone bodies in the fed state. Hepatic uptake of butyrate had ceased, however, by 4–6 days of food deprivation, due to cessation of gut output. At this stage, therefore, non-esterified fatty acids, whose uptake increased (non-significantly) during food deprivation, must have been the main precursor for hepatic ketogenesis.

Table 5 lists the concentrations of the ketone bodies that were found in the arterial blood of the two catheterized non-lactating cows during feeding and during food deprivation. In the fed state, the concentrations of both hydroxybutyrate and acetoacetate were higher than in the jugular blood of the fed cows in Expt. A (Table 1). Food deprivation caused an apparent doubling of the arterial concentration of acetoacetate, but had no effect on that of hydroxybutyrate. Consequently, total ketone-body concentration was little altered. In the food-deprived state there was much less difference between jugular and arterial ketone-body concentrations, as observed in Expts. A and B respectively, than there had been in the fed state.

The finding that net hepatic output of ketone bodies does increase in non-lactating cows during food deprivation is consistent with the nature of the changes in hepatic metabolite concentration that were observed in the food-deprived non-lactating cows studied in Expt. A (Tables 2 and 3). Furthermore, the additional observation that net splanchnic output of ketone bodies did not increase during food deprivation helps to account for the resistance of the non-lactating cows to the development of hyperketonaemia.

Nevertheless, ketone-body concentrations did increase somewhat in the jugular blood during the food deprivation (Table 1), and it becomes necessary to explain how this increase came about if there was no increase in splanchnic output of ketone bodies. One possibility is that the utilization of ketone bodies by peripheral tissues decreases during food deprivation. Support for the possibility of a decrease in the capacity of peripheral tissues to oxidize ketone bodies under these circumstances in provided by the observation of Varnam *et al.* (1978) that there is a decrease in the activities of 3-oxo acid CoA-transferase (EC 2.8.3.5) and acetyl-CoA acetyltransferase (EC 2.3.1.9) in kidney cortex and cardiac muscle when non-pregnant ewes are deprived of food for 4 days. A decrease in peripheral utilization of ketone bodies would also help to explain the differences that were observed between the concentrations of ketone bodies in jugular blood in Expt. A (Table 1) and arterial blood in Expt. B (Table 5). Thus, in the fed state, the jugular concentration would be expected to be lower than the arterial concentration, owing to utilization by the tissues of the head. During food deprivation, the arteriovenous concentration difference across the head would decrease in step with decrease in utilization and the jugular concentration of ketone bodies would rise towards the value of the arterial blood. This is, in fact, what was observed (cf. Tables 1 and 5). The suggestion that a decrease in

Table 5. *Effect of food deprivation on the arterial concentrations of the ketone bodies in the catheterized non-lactating cows (Expt. B)*

Concentrations are expressed as mM in whole blood. The values for the fed state are the means of six observations and those for the food-deprived state are the means of four observations (see the Experimental section).

Metabolite	Fed	Food-deprived	S.E.M.
D-3-Hydroxybutyrate	0.61	0.60	0.19
Acetoacetate	0.03	0.07	0.07
Total ketone bodies	0.64	0.67	0.25

utilization of ketone bodies may have occurred conflicts, however, with the conclusion of Bergman & Kon (1964) that in ewes utilization does not limit ketone-body turnover until the circulating concentration has reached 2mM.

An important element in the increase in hepatic ketogenesis that occurred in the catheterized cows during food deprivation was the switch from uptake to output of acetoacetate. None of the changes in hepatic metabolite concentration that were observed in the present study seems to be clearly implicated in this phenomenon. A preponderance in output of hydroxybutyrate over that of acetoacetate in both the fed and food-deprived states is surprising in view of the low activity of D-3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) in bovine liver (e.g. Baird *et al.*, 1968). In the cows the ratio of splanchnic output of hydroxybutyrate to that of acetoacetate was still 10:1 during food deprivation. This compares with a ratio of 1:1 in humans deprived of food for 3 days (Garber *et al.*, 1974).

Hepatic metabolite concentrations and hepatic ketogenesis

The fact that there was an increase in output of ketone bodies from the liver but not from the splanchnic bed during food deprivation could account for the gradient in ketone-body concentration between liver and blood in the food-deprived non-lactating cows (Tables 1 and 2). The absence of any such gradient in the food-deprived lactating cows of Baird *et al.* (1972) might then be explained if hepatic output of ketone bodies in these animals increased to a much greater extent than in the non-lactating cows in the present study, the increase in hepatic ketogenesis being such as to ensure that ketogenesis from the splanchnic bed as a whole was markedly greater during food deprivation than in the fed state. This would also explain why the lactating cows became strongly hyperketonaemic. If the above assumptions are correct, then it is clear that neither an increase in cytoplasmic reduction (Wieland *et al.*, 1964) nor a decrease in energy charge (Atkinson, 1968) is likely to be involved in regulating the magnitude of hepatic ketogenesis, since these metabolic changes were as great in the food-deprived non-lactating cows in the present experiment as in the food-deprived cows of Baird *et al.* (1972) (Tables 2 and 3). The hepatic concentrations of glycogen and of several intermediates of the Embden–Meyerhof pathway and the tricarboxylic acid cycle did not fall to such low values in the non-lactating cows as in the lactating cows (Table 2). This suggests that the concentration of these compounds might be of more importance in determining the rate of hepatic ketogenesis. Such a conclusion would be consistent with the view of McGarry and co-workers (e.g. McGarry *et al.*, 1976, 1977) that in the rat there is a reciprocal relationship between the hepatic content of glycogen, and possibly also of intermediates of carbohydrate metabolism, and the rate of hepatic oxidation of non-esterified fatty acids. In the work carried out in this laboratory on dairy cows a reciprocal logarithmic relationship between the liver content of glycogen and of total ketone bodies has been consistently observed (Fig. 2). Herriman & Heitzman (1978) provide a similar, but non-logarithmic, plot relating hepatic citrate and hydroxybutyrate content in sheep and cows. The behaviour of 2-oxoglutarate did not conform to the above pattern, however, since the concentration of this compound fell to a lower value in the non-lactating cows than in the lactating cows (Table 2). The concentrations of glycerol 1-phosphate and glucose were also higher in the non-lactating cows than in the lactating cows during food deprivation (Table 2). There compounds could play an antiketogenic role by increasing the rate of esterification of fatty acids (e.g. McGarry & Foster, 1972).

As Table 4 shows, hepatic uptake of non-esterified fatty acids in the non-lactating cows in Expt. B was 4 times as much on days 4 and 6 of food deprivation as in the fed state. At the same time, hepatic output

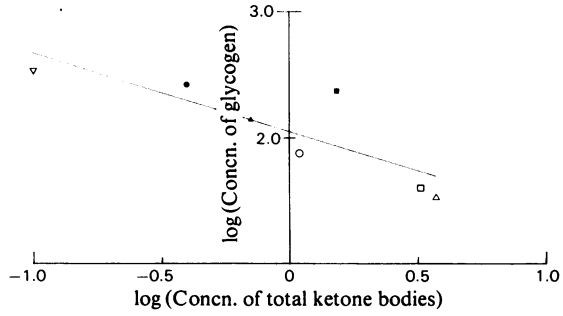

Fig. 2. *Relationship between the concentrations of glycogen and of total ketone bodies in the livers of dairy cows*
Concentration of total ketone bodies (hydroxybutyrate + acetoacetate) is expressed as μmol/g wet wt. of liver and that of glycogen as μmol of glucose equivalent/g wet wt. of liver. Each point represents the mean value obtained for cows in one particular state. △, Food-deprived lactating cows (Baird *et al.*, 1972); □, spontaneously ketotic lactating cows (Baird & Heitzman, 1971); ○, food-deprived non-lactating cows (Table 4); ▲, fed lactating cows (Baird & Heitzman, 1970); ■, spontaneously ketotic lactating cows treated with a glucocorticoid (Baird & Heitzman, 1971); ●, fed non-lactating cows (Table 4); ▽, food-deprived lactating cows treated with glucose (Treacher *et al.*, 1976). The equation of the line is $y = -0.63x + 2.05$. The correlation coefficient $= -0.845$ ($P < 0.02$).

of ketone bodies was only twice as great. This could indicate that food deprivation had not in fact elicited any change in hepatic intermediary metabolism that would increase the proportion of assimilated non-esterified fatty acid that was converted into ketone bodies (cf. Williamson *et al.*, 1969; Bieberdorf *et al.*, 1970). If that were so, then the increase in hepatic ketogenesis might be due solely to increase in the supply of non-esterified fatty acid. The situation is complicated by the fact that at least a portion of the hepatic ketone-body production in the fed state is probably derived from butyrate. However, even if the butyrate taken up by the liver in the fed animals were converted quantitatively into ketone bodies, apparent ketogenesis from non-esterified fatty acid alone would still only be 4 times greater on day 6 of food deprivation than during feeding. This point clearly requires further elucidation.

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