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Metabolic and hormonal aspects of bovine ketosis and pregnancy toxaemia in the ewe

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Metabolic fuels of the ruminant consist mainly of volatile fatty acids produced in the rumen by microbial digestion of dietary polysaccharides and amino acids derived from dietary protein. As almost no glucose is obtained directly from its food, any specific requirements for this sugar are met by synthesizing it from non-carbohydrate sources: principally propionate and the glucogenic amino acids.

Thus, in a physiological emergency, when metabolic pathways for gluconeogenesis are overtaxed, the ruminant may be placed in a state of carbohydrate insufficiency and ketosis develops. This is probably what happens in bovine ketosis and ovine pregnancy toxaemia (see recent reviews by Schultz (1968) and Reid (1968)) and arises because the cow at peak lactation transforms large quantities of blood glucose into the lactose of milk, and the pregnant ewe, particularly when bearing multiple foetuses, diverts blood glucose to support foetal growth and development.

In either case, a stage is eventually reached when affected animals go off their food and the metabolic predicament is aggravated. The cow is able to adapt to the situation by simply dropping its daily milk yield but, in contrast, the pregnant ewe has a limited capacity for adaptation and either aborts its foetuses and eventually recovers or develops a fatal hypoglycaemic ketosis.

Usually it is difficult to obtain suitable field cases for laboratory investigations of the metabolism of ketotic animals as the subjects of study are good milkers or come from valuable breeding stock. Therefore, a considerable amount of published information relates to experimentally induced ketosis. Ketosis has been induced in high-yielding cows by administering growth hormone (Kronfeld, 1965*a*) or thyroxine (Hibbitt, 1964; Hibbitt & Baird, 1967) daily at about the time of peak lactation. This ensured good milk production and exerted the required strain on the metabolic

resources of the cow but such treatment undoubtedly upsets the normal hormonal balance at the time of the experiment. Pregnancy toxaemia has also been induced in ewes bearing twin or triplet foetuses (Reid, 1960; Reid & Mills, 1962; Saba, Burns, Cunningham, Hebert & Patterson, 1966). Undernutrition in late pregnancy imposed the initial restriction on the availability of glucogenic substrates at a time when foetal energy demands were greatest. However, this alone was insufficient to induce clinical pregnancy toxaemia, and 'stress' in the form of adverse weather conditions or the psychological stress of an environmental change was an essential if ill-defined contributory factor.

'Caloric homoeostasis' in ruminant ketosis

The primary response to glucose insufficiency appears to be a stimulation of depot fat mobilization, non-esterified fatty acids (NEFA) released into the plasma providing tissues of the body with an alternative source of energy. This physiological reaction was termed 'caloric homoeostasis' by Fredrickson & Gordon (1958). When gluconeogenesis fails to keep up with the demand for glucose, the subsequent ketogenesis may be regarded as a secondary form of 'caloric homoeostasis'.

Fat mobilization. The inverse relationship between plasma glucose and NEFA concentrations, which was first described in man by Dole (1956) and Gordon & Cherkes (1956) has also been observed in cows (Adler & Wertheimer, 1962; Kronfeld, 1965*b*) and sheep (Annison, 1960). Depending upon the availability of glucose, the equilibrium in the adipose tissue between neutral fat synthesis and lipolysis shifts, releasing less or more NEFA into the circulation. The diurnal variation in plasma NEFA concentration correlates with feeding habits in sheep and the plasma NEFA concentration can be regarded as a sensitive index of its nutritional status (Russel, Doney & Reid, 1967). Increasing the availability of glucose, e.g. by an intravenous injection, quickly depresses the rate of depot fat mobilization in normal fasted cows (Kronfeld, 1965*b*) and sheep (Annison, 1960; Patterson, 1966). The utilization of glucose by adipose tissue in the starving sheep is facilitated by insulin (Patterson, 1966) but the fact that the inhibitory effect of glucose on fat mobilization was not abolished by the simultaneous administration of an anti-insulin serum (Cunningham & Patterson, unpublished results) suggests that insulin is not a limiting factor.

Relative undernutrition or glucose insufficiency plays an important part in the aetiology of bovine ketosis and ovine pregnancy toxaemia and amongst the first biochemical changes there is an increase in the plasma concentration of NEFA. As this occurs before either hypoglycaemia or hyperketonaemia is detected it seems probable that, in the earliest stages, glucose utilization by adipose tissue is impaired. In pregnancy toxaemia, for example, Reid & Hogan (1959) suggested that diminished glucose utilization was related to increased adrenocortical activity, and Bassett (1963) confirmed that injected cortisol reduced glucose tolerance in ewes. However, in our own experiments on induced pregnancy toxaemia we found no increase in plasma cortisol levels (Saba *et al.* 1966). Whatever the precise endocrine basis, it is well known that stressful situations capable of precipitating pregnancy toxaemia in the starving ewe (excitement, severe weather conditions, environmental change, etc.)

also stimulate depot fat mobilization. Glucose utilization is probably impaired under these conditions, glucose tolerance being inversely related to depot fat mobilization in sheep (Patterson & Cunningham, unpublished observations) and in man (Hollobaugh, Tzagournis, Folk, Kruger & Hamwi, 1968).

Ketogenesis. In fed cows and sheep β-hydroxybutyrate is formed from dietary butyrate in the wall of the reticulo-rumen, and in the lactating cow acetoacetate is synthesized in the udder. The former source disappears on starvation and is at first supplemented and then replaced by hepatic ketogenesis as fasting continues. Ketone bodies (β-hydroxybutyrate, acetoacetate and acetone) come into prominence as metabolic fuels at this stage when glucose is either unavailable or in short supply. In fact, ketone bodies can be readily oxidized by skeletal muscle (Randle, Garland, Hales & Newsholme, 1963) and even preferentially utilized by cardiac muscle (Williamson & Krebs, 1961).

Hepatic ketogenesis is initiated by the condensation of two acetyl-CoA molecules. The availability of this substrate depends upon the balance of the pathways for its formation and its utilization and determines whether ketone bodies are formed in greater or lesser amounts. Excessive fat mobilization yields long-chain fatty acids that can be oxidized to form large amounts of acetyl-CoA, which unless oxidized in the tricarboxylic acid (TCA) cycle or utilized for lipid synthesis, are diverted to form ketone bodies. Thus, it has been shown that the liver of ewes suffering from pregnancy toxaemia forms ketone bodies (mainly β-hydroxybutyrate) at a rate dependent upon the plasma NEFA concentration (Patterson, 1966), and recent work by Leng & West (1969) has shown that ketone bodies will arise entirely from ^{14}C-labelled NEFA during starvation.

While the intravenous administration of glucose immediately suppresses depot fat mobilization in the starving cow and sheep, it only slowly diminishes the rate of ketogenesis. Daily glucose treatment of spontaneously ketotic cows had a more rapid effect on fat mobilization than on production of ketone bodies (Baird, Hibbitt, Hunter, Lund, Stubbs & Krebs, 1968) and intravenous glucose failed to reduce the concentration of blood ketones in cases of induced ovine pregnancy toxaemia (Reid, 1968). These results emphasize the impairment of glucose utilization in ruminant ketosis. The effectiveness of glucose treatment probably also differs as between bovine ketosis and pregnancy toxaemia, the former being more responsive.

Although glucogenic substrates may replace glucose in treating ketosis (Krebs, 1966) glucose itself is specifically required to prevent the appearance of clinical symptoms of pregnancy toxaemia in ewes (McClymont & Setchell, 1955) and the 'nervous' form of bovine ketosis as it is preferentially metabolized by nervous tissues.

The relationship between gluconeogenesis and ketogenesis

Disturbance of the balance of hepatic acetyl-CoA production and utilization is fundamental to the problem of ruminant ketosis. Oxidation of acetyl-CoA in the TCA cycle is initially dependent upon the availability of mitochondrial oxaloacetate (OAA) for the citrate synthase reaction. When insufficient OAA is available, acetyl-CoA tends to be diverted from oxidation to ketogenesis. And, with the inhibition

of fatty acid synthesis that occurs in ovine pregnancy toxaemia and bovine ketosis, this tendency is enhanced (see Kronfeld, 1961).

Another important factor is the link between OAA utilization in gluconeogenesis and ketone body formation (Krebs, 1966). Wherever the metabolic pathways for gluconeogenesis are stimulated OAA is preferentially used for glucose synthesis in the cytoplasm and, in diverting it from the mitochondrial TCA cycle, acetyl-CoA is permitted to form ketone bodies instead of citrate. Evidence in support of this theory has been advanced by Hibbitt & Baird (1967) who studied hormonally induced cases of bovine ketosis and by Baird *et al.* (1968) using spontaneously occurring cases. Although Ballard, Hanson, Kronfeld & Raggi (1968) did not observe either a decreased concentration of liver mitochondrial OAA, or the detailed metabolic changes predicted by Krebs (1966), they did not dispute the link between enhanced gluconeogenesis and ketogenesis.

There are probably as yet undisclosed metabolic differences between starvation ketosis and the clinical conditions of bovine ketosis and ovine pregnancy toxaemia. Thus, Ballard *et al.* (1968) reported that gluconeogenesis is depressed in the starving cow but not in cases of clinical bovine ketosis. On the other hand, in our experiments on induced pregnancy toxaemia, starvation for up to 6 days failed to produce hypoglycaemia in pregnant ewes (unpublished findings), indicating the existence of very adequate glucogenesis but, in ewes developing clinical pregnancy toxaemia, hypoglycaemia developed within a day or so of induction (Saba *et al.* 1966). As glucose utilization is already impaired where depot fat is rapidly mobilized, this result suggests that glucose synthesis had failed.

Liver lipid metabolism in ruminant ketosis

Fat is deposited in the liver of ketotic ruminants and much attention has naturally been given to hepatic lipid metabolism. It has long been known that this lipid is mobilized from the adipose tissue depots in starving sheep (Fraser, Godden, Snook & Thomson, 1939) and the quantity of fat deposited has been found to correlate with the plasma NEFA concentration in experimental ovine pregnancy toxaemia (Patterson, 1966). With unaltered plasma concentrations of triglyceride, it has been concluded that lipid accumulation is not associated with a decreased output of liver lipoprotein (Patterson, Burns, Cunningham, Hebert & Saba, 1964; Reid, 1968). However, it has been shown by McCarthy, Porter & Griel (1968) that blood lipid transport is seriously impaired in severe bovine ketosis. As the livers from undernourished ewes and those suffering from pregnancy toxaemia have decreased levels of α-glycerol phosphate dehydrogenase (Patterson, 1964*a*), triglyceride synthesis cannot involve the utilization of triosephosphate. In all probability, glycerol, released from adipose tissue together with mobilized NEFA's, is phosphorylated in the glycerol kinase reaction.

At the same time as triglyceride is formed in the liver from plasma NEFA, new synthesis of fatty acid from acetate is inhibited in bovine ketosis (Hanson & Ballard, 1967; Ballard *et al.* 1968) and in pregnancy toxaemia (Patterson, 1966). Fatty acid synthesis is also inhibited in the liver of fat-fed rats (Bortz, Abraham & Chaikoff,

1962; Patterson, 1964*c*) and it is possible that, whenever fat is deposited in the liver, acetyl-CoA carboxylation is specifically inhibited. As citrate is required to activate this rate-limiting reaction, lipogenesis would additionally be inhibited by low liver concentrations of this metabolite and, at least in bovine ketosis, this condition does obtain (Ballard *et al.* 1968).

Inhibition of hepatic lipogenesis is likely to involve more than one factor and a deficiency of hepatic $NADPH_2$, the reduced nucleotide specifically involved in fatty acid synthesis, has been considered possible but not conclusively proved (Ballard *et al.* 1968; Patterson, 1963, 1964*a*). Nevertheless, Kronfeld & Raggi (1964) concluded that this nucleotide deficiency was involved in the inhibition of lipogenesis in the udders of ketotic cows.

While there is no overall shortage of liver $NADPH_2$ in the undernourished ketotic ewe, formation of this reduced nucleotide in the hexose monophosphate oxidative pathway is probably impaired. An almost complete blockage occurs at 6-phosphogluconate (Patterson, 1964*a*,*b*). These enzyme changes are apparently less pronounced in starved or spontaneously ketotic cows (Ballard *et al.* 1968).

Endocrine control of metabolism in ruminant ketosis

The primary response to glucose insufficiency has been described above largely as a mass-action effect in the adipose tissue resulting in the release of NEFA. However, it is probable that in the onset of ruminant ketosis, hormonal control is exerted to produce the glucose insufficiency itself, or to exaggerate what is a physiological tendency in the heavily pregnant ewe or in the cow at peak lactation. It may also operate in such a way as to modify the response to the acute state of calorie deficiency, e.g. by exerting control of fat mobilization or gluconeogenesis or both.

(a) *Insulin.* Insulin facilitates the uptake of glucose by the various tissues of the body and a state of insulin deficiency (diabetes) is similar, in certain respects, to ruminant ketosis. However, although there is a relative insulin deficiency, in pregnancy toxaemia for example (Saba *et al.* 1966), this appears to be a response to the onset of hypoglycaemia rather than the cause of impaired glucose utilization and depot fat mobilization. Thus, whereas glucose insufficiency in diabetes is related to decreased utilization caused by insulin deficiency and is characterized by hyperglycaemia, the same condition in ruminant ketosis results from a physiological drain on a limited supply of glucose and probably results from the lowered blood glucose concentration itself.

(b) *Adrenalin.* Adrenalin can stimulate fat mobilization in sheep (Annison, 1960) and cows (Kronfeld, 1965*a*,*b*) and while 'stress' factors may, theoretically, result in an increased secretion of adrenalin, there is no evidence for an enhanced adrenal output of catecholamines in experimentally induced pregnancy toxaemia (Saba *et al.* 1966).

(c) *Hormones of the adrenal cortex.* Adrenocortical hormones have been implicated in the aetiology of ruminant ketosis from time to time. Published observations on levels of plasma cortisol in bovine ketosis, however, are conflicting (Robertson, Lennon, Bailey & Mixner, 1957; Horrocks & Paterson, 1960) and studies of the

urinary excretion of steroids by Heitzman (1967) and Heitzman & Hibbitt (1967) suggest that there were no alterations in pituitary–adrenal function. In their studies on experimentally induced pregnancy toxaemia, Reid and co-workers concluded that hyperadrenocortical activity accompanies the 'stress' that is known to precipitate the clinical condition (see Reid, 1968), but our experiments did not confirm this (Saba *et al.* 1966). Indeed, these experiments showed that the daily administration of corticotrophin (ACTH) prevented the appearance of the clinical condition, suggesting, if anything, an adrenocortical deficiency. A similar conclusion was reached by Pinkiewicz (1963) when examining clinical cases of bovine ketosis. ACTH treatment reduces milk yield (Brush, 1960; Campbell, Davey, McDowall, Wilson & Munford, 1964), raises the blood sugar level and thereby alleviates clinical symptoms of bovine ketosis (Shaw, 1956). In correcting hypoglycaemia, ACTH is believed to activate gluconeogenesis by inducing the formation of increased amounts of glucocorticoid in the adrenals. In preventing the onset of pregnancy toxaemia, however, the abortifacient effect of ACTH has to be considered as an additional mechanism (Saba *et al.* 1966).

(d) *Fat mobilizing substance* (*FMS*). The gross metabolic changes produced in rodents treated with FMS are strikingly similar to those seen in bovine ketosis and pregnancy toxaemia, namely: hypoglycaemia, raised plasma NEFA concentrations, hyperketonaemia, deposition of liver fat and loss of body-weight. FMS which apparently acts by stimulating lipolysis in the adipose tissue without affecting fatty acid esterification (Cahill, Pawan & Chalmers, 1961) has been isolated from the urine of fasting human subjects and its excretion is dependent upon a functional pituitary gland (Chalmers, Kekwick, Pawan & Smith, 1958). A similar compound was reported to have been isolated from sheep urine (Chalmers *et al.* 1958; Kekwick & Pawan, 1967) and for this reason we attempted to isolate this substance from urine of starving cows and sheep with the object of investigating the possible role of FMS in regulating metabolism in ruminant ketosis.

More than fifty 24 h urine specimens from starving cows and sheep and from a single case of spontaneous bovine ketosis were examined, by exactly the same procedure as that of Chalmers *et al.* (1958), and found to be without FMS activity. The only property ascribable to any active material isolated (containing about 75% protein) was a hypoglycaemic effect. The subcutaneous administration of about 5 mg protein, representing 1–5% of a fasting sheep's daily output, lowered mouse blood glucose concentrations by 25–45/mg 100 ml after 3·5 h.

The identity of human FMS is still uncertain and the hypoglycaemic substance isolated from cow and sheep urine may well be part of a labile ruminant FMS or even a fragment of some other polypeptide hormone.

(e) *Growth hormone.* It has been suggested that hypersecretion of this anterior pituitary hormone is a factor in the aetiology of bovine ketosis (Seekles, 1948; Kronfeld, 1963, 1965*a*), and pregnancy toxaemia (Reid, 1968). As growth hormone-induced ketosis of non-ruminants is associated with increased fat mobilization, increased fat oxidation, decreased lipogenesis and increased hepatic ketogenesis (Knobil & Hotchkiss, 1964), it is conceivable that excessive secretion of this hormone

might initiate the development of ruminant ketosis. Furthermore, the secretion of growth hormone impairs glucose utilization (Hollobaugh *et al.* 1968) and is stimulated by hypoglycaemia (Roth, Glick, Yalow & Berson, 1963; Knobil & Hotchkiss, 1964). It is thus possible that the physiological emergencies causing hypoglycaemia in ruminant ketosis might, therefore, indirectly trigger off growth hormone secretion in susceptible animals. This aspect of ruminant ketosis is now under consideration.

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Kale anaemia

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The two main types of kale grown in Britain, marrowstem and thousandhead, both belong to the species *Brassica oleracea* L. Marrowstem is a thick-stemmed plant, 1–1·5 m tall, whereas thousandhead has a higher ratio of leaf to stem and is a shorter plant. Kale is sown in late spring and normally harvested from October to February, thousandhead being the more resistant to frost. The crop yields about 50 000 kg green matter per hectare (7000 kg dry matter) and is either consumed *in situ* or cut and carried to housed animals. Cattle are rarely fed on kale alone, but sheep may be; when kale is grazed its consumption is not closely controlled. Kale is grown widely in Britain and north Europe generally, and also in New Zealand.

In the UK, the area devoted to kales increased from 85 000 ha in 1946 to a maximum of 160 000 ha in 1960, but has since declined rapidly to 86 000 ha in 1966. This decline is partly due to problems associated with the harvesting of the crop, but may be partly attributable to an increasing incidence of so-called kale poisoning. This condition had been seen previously in Germany when kale production was increased during the Second World War (Rosenburger, 1950).

Field cases of kale anaemia

Only a brief account will be given here of the clinical signs of the disease as it occurs on farms; a full description has been given by Clegg (1966). The animals most commonly affected are dairy cows. After 2–4 weeks of kale feeding, they show weakness, inappetance and, frequently, haemoglobinuria. Milk yield falls, pulse and respiration rates increase and affected animals may die. The main haematological changes are a fall in haemoglobin concentration, from the normal 11 g/100 ml to less than 3 g/100 ml in some cases, and the appearance of Heinz Ehrlich bodies in the red cells. Clegg (1966) describes the changes found *post mortem*. Other *Brassica* species, such as rape, also cause anaemia. The incidence of the condition is unknown. Connold (1952) questioned ninety-four farmers in south-west England about their experience of kale and rape, and reported 'four or five cases of rape poisoning'.

Brassica species are known to cause other disorders, particularly goitre and reproductive disorders (Williams, Hill & Alderman, 1965).

Experimental studies

Kale anaemia has been studied experimentally in Germany (Steger, Piatkowski,