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VARIATIONS IN SOME PLASMA METABOLITES FROM NEOGLUCOGENESIS AND KETOGENESIS IN PREGNANT EWES IN RELATION TO DIET

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SUMMARY

Four groups of eight *Charmoises* ewes weighing between 50 and 60 kgs were given the following diets from ninth week of gestation onwards :

- high-low group : high level of feeding until the 15th week (hay *ad libitum* plus 0.8 kg concentrates) then low level until dropping (0.65 kg hay),
- low-high group : low then high levels of feeding,
- low group : restricted feeding during the whole pregnancy (0.65 kg then 0.8 kg hay),
- control group : increasing level of feeding during pregnancy (hay *ad libitum* plus 0.3 kg then 0.5 kg concentrates).

The plasma concentrations of glucose, alanine and urea were measured in order to determine the importance of glucogenic precursors such as alanine. The links between lipid and acetate metabolism were studied by determination of the plasma levels of free fatty acids, 3-hydroxybutyric acid and acetic acid.

None of the control and low-high ewes showed any significant increase in 3-hydroxybutyric acid or any severe hypoglycemia. In the high-low and low groups twin-pregnant ewes only showed during the final month of pregnancy the biochemical, but not the clinical, symptoms of acetone-mia.

Ketogenesis occurs to any large extent only if the free fatty acid level is high and the level of glucose precursors is low. Thus, it is the differences of glucose drainage and neoglucogenesis, as well as lipomobilization, which distinguish ewes bearing one or two fetuses.

The hypothesis that the metabolism of free fatty acids taken up by the liver is closely dependent on neoglucogenesis is suggested. This would concern re-esterification, mitochondrial penetration (activity of the carnitine acyl transferase system) and probably the intensity of β oxydation and activity of the Krebs cycle.

Parallel increases in the plasma concentrations of free fatty acids, 3-hydroxybutyric acids and acetic acid occur. There is certainly an increase in the production of hepatic endogenous acetate from acetyl CoA and there is perhaps also difficulty in the peripheral use of acetate and 3-hydroxybutyrate as a result of hypoglycemia.

INTRODUCTION

At the end of pregnancy, especially when they carry two foetuses, ewes show certain metabolic irregularities (hypoglycemia, hyperketonemia, increase in free fatty acids in the plasma) which may become pathogenic if the animal is under stress (REID, 1968).

The energetic needs of the foetus are mainly supplied by glucose oxidation and two foetuses can drain more than 50 p. 100 of the glucose produced by the mother during the last 6 weeks of the gestation (KRONFELD, 1970 ; CHRISTENSEN and PRIOR, 1974). Thus its glucose and amino acid requirements are considerable, and must be covered by a reduced intake volume since ingestion is diminished due to the volume occupied by the foetuses and by the abdominal fat (FORBES, 1970) and by the action of and endocrine factors (ROBINSON, 1957 ; FORBES and ROOK, 1970). In addition, heat production increases towards the end of pregnancy (MCGRAHAM, 1964).

Mobilization of lipids following energy deficiency or stress (or both factors together) will give rise to ketogenesis, the level of which depends on the severity of hypoglycemia (ROBINSON, FRASER and BENNETT, 1971). Stresses during the last two months of pregnancy can be avoided by maintenance of the animal in a stable, favorable environment, but the nutrition of the ewe remains important : errors in the diet of the flock can give animals which are too fat at the beginning of pregnancy or poorly fed at the end.

The variations in metabolites such as glucose, free fatty acids and ketone bodies are generally well known for different diets (REID and HINKS, 1962 ; PATTERSON *et al.*, 1964). This study was to determine the importance of glucogenic precursors such as alanine and also the relation between lipid and acetate metabolism.

EXPERIMENTAL TECHNIQUES

Animal maintenance

2 to 4 year-old *Charmoise* ewes weighing between 50 and 60 kg were divided, during the second month of pregnancy, into 4 groups of 8 animals which were housed in adjacent corrals. The experience was performed with two dietary periods : beginning of pregnancy (9th to 15th week) and end of pregnancy (15th to 20th week).

Control group (C) : natural grassland hay *ad libitum* + 0.3 kg concentrates at the beginning of pregnancy and 0.5 kg at the end of pregnancy.

High-low group (H-L) : hay *ad libitum* + 0.8 kg concentrates, then 0.65 kg hay only.

Low-high group (L-H) : 0.65 kg hay, then hay *ad libitum* and 0.8 kg concentrates.

Low-low group (L-L) : 0.65 kg hay, then 0.80 kg hay.

The animals in all groups had straw *ad libitum*. The concentrates had the following composition : 30 p. 100 maize, 30 p. 100 barley, 20 p. 100 oats, 20 p. 100 peanut cake. The percentage of protein was 20 p. 100 of dry matter for the concentrate and 9.5 p. 100 for hay ; crude-cellulose was 34 p. 100 of dry matter of hay.

Sampling in the jugular vein was done at 9 a.m. before the single meal of the day, after 61, 77, 96, 109, 126, 133 days of pregnancy and the last week before parturition.

Biochemical techniques

Venous blood was obtained from the jugular vein and immediately centrifuged in heparinized microtubes and plasma are frozen at -20°C . Measurements of glucose were made by hexokinase (SLEIN, 1963) alanine by alanine dehydrogenase (WILLIAMSON, 1970) and 3-hydroxybutyrate by 3-hydroxybutyrate dehydrogenase (WILLIAMSON and MELLANBY, 1963) after deproteinisation by perchloric acid 0.6 M 1 : 2, v/v). The free fatty acids after extraction by ethanol and evaporation were dissolved in chloroform/heptane 4 : 3 v/v according to Ho and MENG (1969) and measured colorimetrically in the form of copper salts. Urea was measured by diacetylmoxine (MARSH, FINGERHUT and KIRSHE, 1965) and plasma acetate by gas chromatography after ethanol extraction (REMESY and DEMIGNE, 1974).

RESULTS

Distribution of lambing in the different groups :

	Non pregnant	Single pregnant	Twin pregnant
C	0	5	3
HL	2	4	2
LH	1	5	2
LL	2	4	1

In the following figures ewes in the LH and LL groups were combined for the first pregnancy period.

The reduction in nutrition was very severe since it did not even correspond to maintenance needs estimated at 25 g MOD/W^{0.75}/day (ROBINSON, FRASER and BENNET, 1971). Assuming a mean value of 400 g MOD/kg of hay, maintenance needs would be 1 250 g hay for ewes weighing 55 kg. The quantities of concentrates for the control and high energy groups almost certainly filled the animals energy requirements except perhaps for the controls at the end of pregnancy.

Glucose (fig. 1)

In the control group there was no evident difference in the glycemia of single (G₁) and twin (G₂) pregnant ewes. Glycemia tended to be reduced at the end of pregnancy except just before dropping when it increased again. This phenomenon was observed with all the nutritional groups. The H-L group, in spite of excessive feeding at the beginning of pregnancy showed large differences between the glycemia of G₁ and G₂ ewes. With change of diet, these differences persist but with low levels of glycemia. In the case of prolonged underfeeding (LL group) the differences were more marked during the last month of gestation. The well-known resistance to hypoglycemia of ruminants cannot be entirely explained by increased utilisation of ketone bodies ; even during pronounced ketonaemia, the cerebral sheep brain uses mainly glucose for its oxydative metabolism (LINDSAY and SETCHELL, 1972).

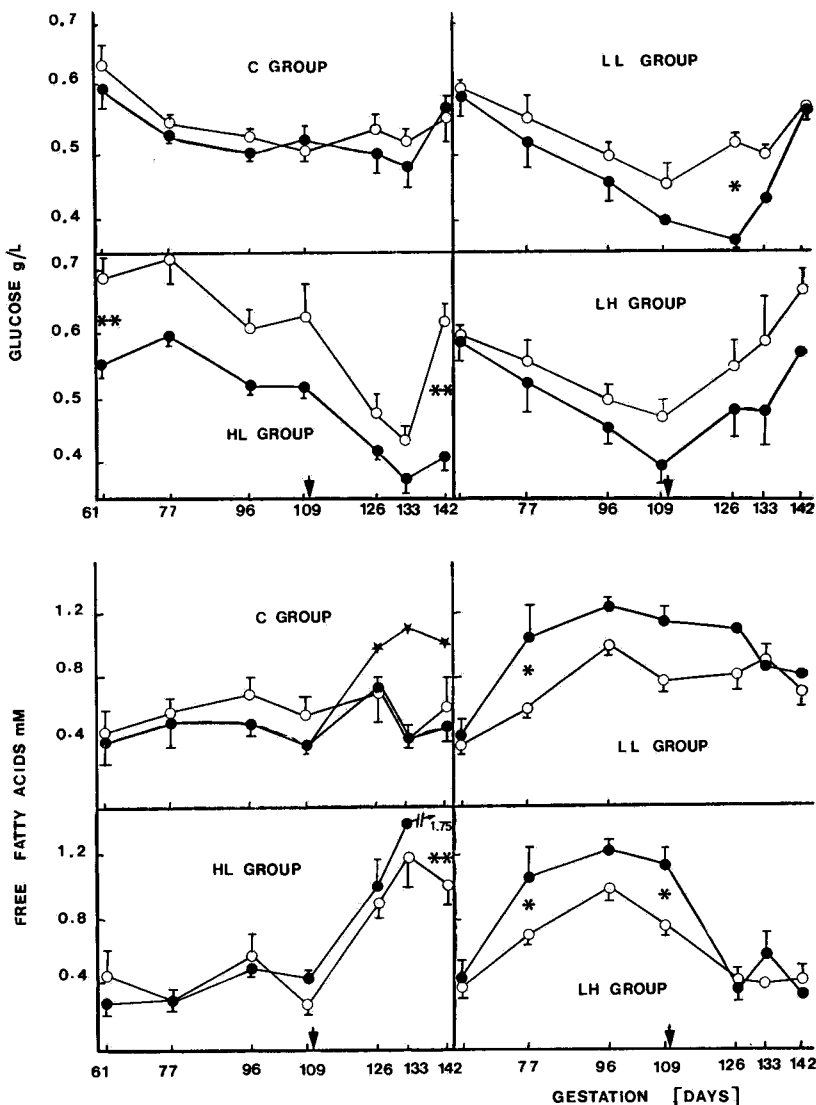


FIG. 1. — Changes in the plasma levels of glucose and free fatty acids during pregnancy for different diets. Each point represents the mean (\pm S. E. M.) for the number of animals given in the lambing results. The arrows indicate change of diet. The sampling dates are given as the mean number of days after mating of the ewes in the group.

- = single pregnant ewes
- = twin pregnant ewes
- ★ = twin pregnant ewe with spontaneous lipomobilization
- * = significant difference between G_1 and G_2 ewes ($P < 0.05$)
- ** = significant difference between G_1 and G_2 ewes ($P < 0.02$)

Modifications du niveau du glucose plasmatique et des acides gras libres pour différents régimes. Chaque point représente la moyenne (\pm l'erreur type) pour le nombre d'animaux indiqué dans le paragraphe résultats (distribution des agnelages). Les flèches indiquent le changement de régime. Les dates de prélèvement représentent le nombre moyen de jours après l'accouplement pour les brebis du groupe.

- = brebis gestantes d'un agneau
- = brebis gestantes de jumeaux
- ★ = cas particulier d'une brebis gestante de jumeaux qui a présenté une lipomobilisation spontanée
- * = différence significative entre les brebis G_1 et G_2 ($P < 0,05$)
- ** = différence significative entre les brebis G_1 et G_2 ($P < 0,02$)

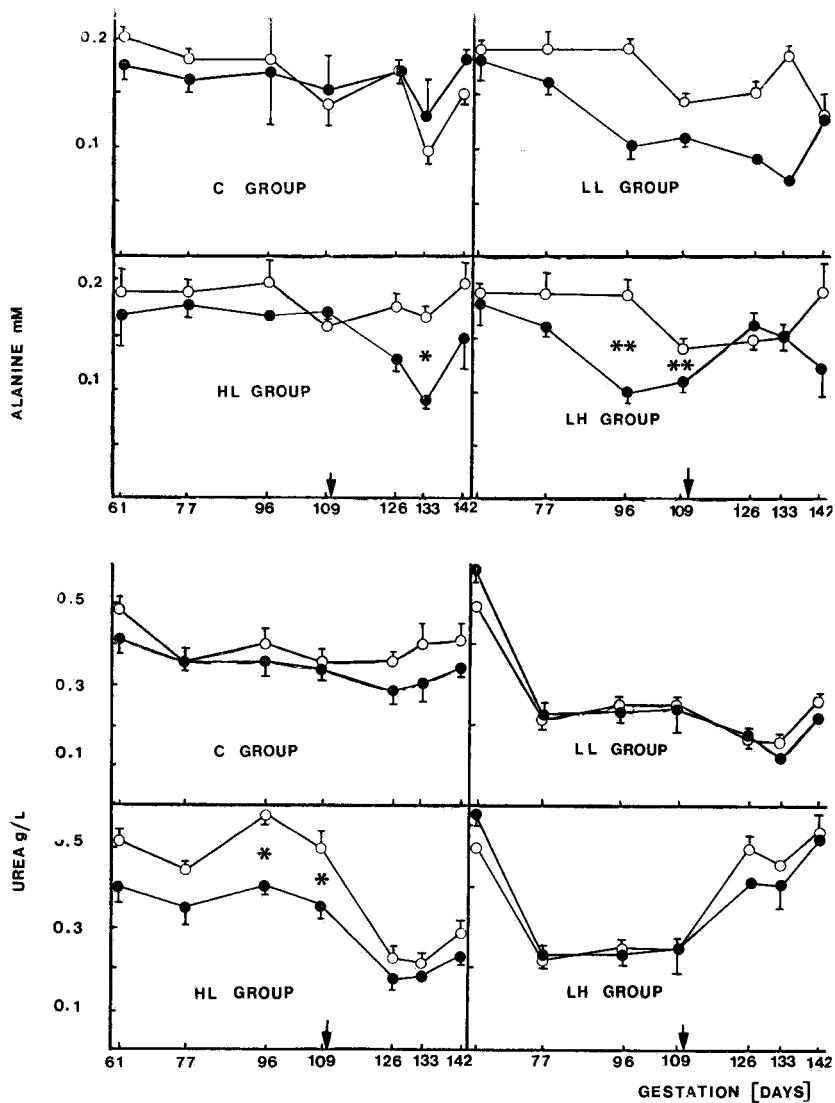


FIG. 2. — Changes in the plasma levels of alanine and urea during pregnancy for different diets (details see figure 1)

Modifications du taux plasmatique d'alanine et d'urée pendant la gestation pour différents régimes (légende voir fig. 1)

Alanine (fig. 2)

Alanine is the most important glucose-forming amino acid (WOLFF and BERGMAN, 1972). In ruminants, since neoglucogenesis is very active and protein catabolism weak as a result of limited absorption of amino acids, alaninemia is lower than in monogastrics (0.5 mM) (FELIG, 1973). With the control group, variations in alanine concentration were slight, in correlation with the level of glycemia. During all the periods of underfeeding (LL group, HL group, 2nd period; LH group, 1st period) alaninemia was sharply reduced in G₂ ewes. The absence of glucose precursors like alanine should be thus responsible for hypoglycemia. However G₁ and G₂ ewes may show differences in glycemia but not in alaninemia (HL group, 1st period) when glycemia is relatively high. Foetal concentrations of alanine are high (HOPKINS, MCFADYEN and YOUNG, 1971); alanine is actively transported to the foetus but transfer down the gradient to the dam may be considered, as the gradient is particularly important for alanine, produced in the foetal metabolism.

Urea (fig. 2)

The effect of pregnancy on the reduction of ureogenesis in well fed ewes was reported by MC GRAHAM (1968) and ROBINSON, SCOTT and FRASER (1973). The level of nutrition always has a marked influence on uremia (LH and LL group). In addition, differences between G₁ and G₂ ewes can be observed resulting from the increased protein requirements in G₂ ewes for foetal growth in non-restricted groups. Whenever hypoalaninemia occurs (G₂ of groups LH and LL) the urea level in the blood is low and there is no difference between G₁ and G₂ ewes. Therefore, alaninemia is a better index of amino acids shortage than uremia.

FFA and ketone bodies (fig. 1 and 3)

In ruminant, 3-hydroxybutyric acid is the main ketone body and its variations are the most representative. As acetoacetic acid could not be immediately determined, its measurement was not made due to unstability of this ketoacid. A prolonged increase in FFA concentration is always evidence of an energy deficiency (DAVIES, JOHNSTON and ROSS, 1971; ROBINSON, FRASER and BENNETT, 1971). Hyperketonemia and hypoglycemia develop more slowly.

In the control group there does not appear to be any energy deficiency at the beginning of pregnancy and only one G₂ ewe showed noticeable lipomobilization. The endocrine state thus appears determining and lipomobilization at the end of pregnancy is a physiological phenomenon rather than evidence of under-feeding. Where there is underfeeding, while lipomobilization is constant (LL group), ketone bodies slowly increase as hypoglycemia and hypoalaninemia develop. Underfeeding at the end of pregnancy of relatively fat animals (HL group, second period) favors maximum lipomobilization but ketogenesis occurs only after the animal has exhausted its reserves of glucose precursors. It is then very active. Lipomobilization appears to increase in relation to hypoglycemia and the size of lipid reserves.

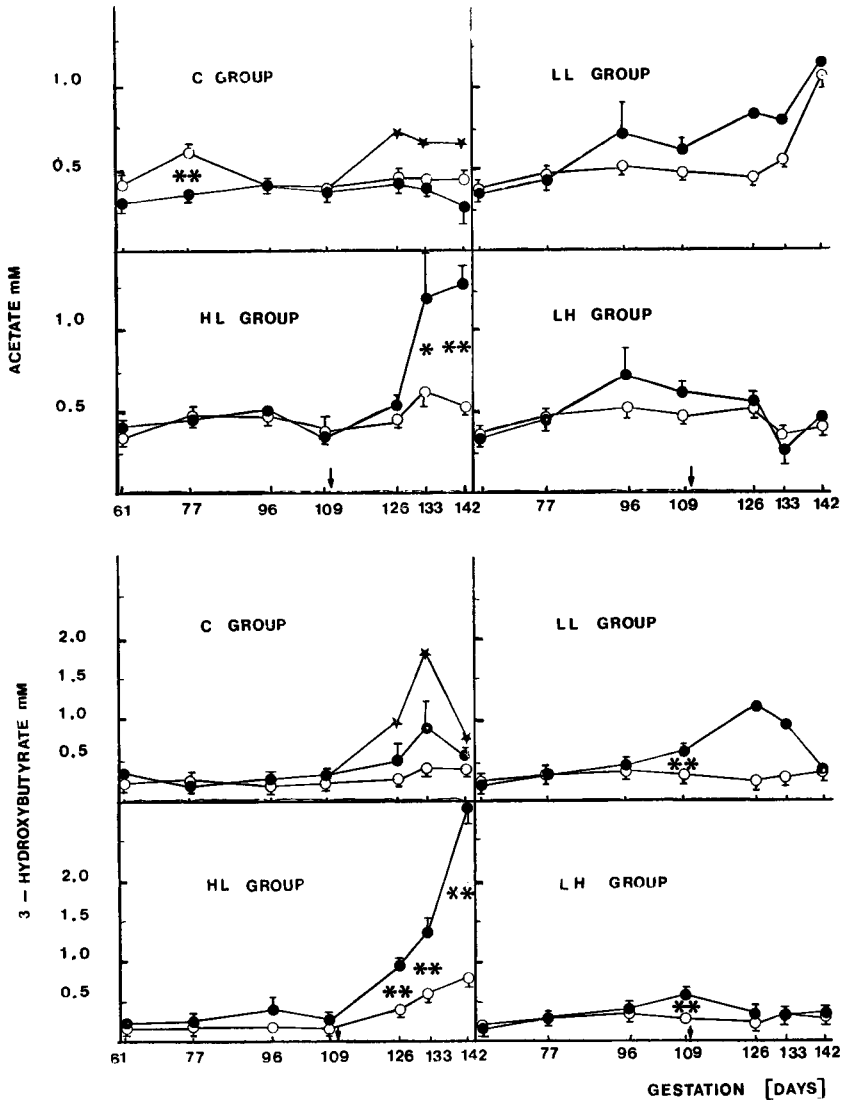


FIG. 3. — Changes in the plasma levels of acetate and 3-hydroxybutyrate for different diets (details see figure 1)

Modifications du taux plasmatique d'acétate et de 3-hydroxybutyrate pour différents régimes (légende voir fig. 1)

Acetate (fig. 3)

The peripheral concentrations reflect a complex equilibrium between the production of acetate, either by fermentation or by endogenous production (which is related to ketone bodies) and its utilisation by the tissues.

In the controls, there was an increase in plasma acetate in the G₂ ewe which showed noticeable lipomobilization. This probably resulted from an increased production of endogenous acetate, which also occurs in G₂ ewes of the HL group at the end of pregnancy. However it is also possible that an increase in acetate was due to poor metabolism induced by a lack of glucose, which is general in G₂ ewes of the LH (1st period) and LL groups. The level of plasma acetate reflects not only rumen production but also the availability of glucose for its utilization. Acetate production is almost certainly higher in animals fed *ad libitum* than in those on a restricted diet.

DISCUSSION

The control of lipomobilization

The combination of stress and fasting cause hypoglycemia and severe hyperketonemia which are responsible for pregnancy toxemia (REID, 1968). Progesterone may determine some economy of glucose in the tissues of the mother, thus favorising lipomobilization as LINDSAY (1973) suggested.

Spontaneous lipomobilization, such as that observed in a G₂ ewe of the control group, are certainly due to endocrine or appetite irregularities. The intensity of lipomobilization varies very considerably with the energy content of the diet and the fatness of the animal. A maximum is obtained in a fat animal on a restricted diet. Animals that are over-fat at the end of pregnancy may show reduced appetite caused by fat accumulation or metabolic (FORBES, 1970) or endocrine factors such as oestrogens (FORBES and ROOK, 1970). Supplementary problems may occur when there is accumulation of fat in the liver. This is quite frequent in pregnant ewes even in the absence of clinical symptoms (LODGE and HEANEY, 1973).

Foetal metabolism

Foetal requirements for energetic metabolism and anabolism are not completely known for every physiological situation, concerning the balance between glucose (plus lactate) and amino acids. According to YOUNG (1975) the ratio of net maternal to foetal transfers of glucose/amino acids is 0.8. A part of glucose (40 p. 100) taken up by the foetus is incorporated in carbon compounds (JAMES *et al.*, 1972), this part is higher (60 p. 100) for amino acids (COMLINE and MESSAGE, 1965). SIMMONS *et al.* (1974) claimed that amino acid catabolism can account for 90 p. 100 of foetal O₂ consumption during prolonged maternal starvation. It is unclear if foetal neoglucogenesis contributes significantly to glucose requirements although glucogenic pathways are active during the last month of gestation (BALLARD and OLIVER, 1965).

Acetate (CHAR and CREASY, 1976), 3-hydroxybutyrate (MORRIS *et al.*, 1974), FFA and glycerol (JAMES, MESCHIA and BATTAGLIA, 1971) makes a poor contribution to foetal metabolism even when maternal blood levels are very high.

Regulation of neoglucogenesis

This regulation acts both on the supply of substrates to the liver and on the activity of limiting enzymes of neoglucogenesis. Pregnant ewes respond to increased glucose requirements by increased neoglucogenesis even when the availability of precursors does not change. The conversion of propionate to glucose normally increases from 46 to 57 p. 100 during pregnancy (according to STEEL and LENG, 1973). However, in the case of under-feeding propionate plays a reduced role in glucose synthesis and neoglucogenesis utilises endogenous substrates. The importance of glycerol increases with lipomobilization although part may be considered with lactate as a form of glucose recycling. No figure is presented for lactate because of the frequent interference of handling stress on the lactacidemia. However, there are clear differences between restricted G₁ and G₂ ewes with lactacidemia of 1.16 ± 0.05 and 0.70 ± 0.09 mM respectively at the end of pregnancy.

The principal substrates of neoglucogenesis are, in reality, amino acids. The measurement of alanine is very important in this respect for it is the principal result of the transformation of branched amino acids in muscle and of glutamine in the intestine (WOLFF and BERGMAN, 1972). The hypoalaninemia which accompany hypoglycemia in G₂ ewes is evidence of the important role of amino acids in neoglucogenesis. Their uptake by the liver is certainly increased by a higher ratio glucagon/insulin (BROCKMAN and BERGMAN, 1975). Since ureogenesis and neoglucogenesis are tightly linked, hypoalaninemia without increased hypouremia indicates that alanine uptake by the liver is increased although peripheral production of this amino acid is not affected. In G₂ ewes peripheral mobilization capacities appear insufficient, probably because the glucocorticoids remain relatively stable. In addition, the activity of urea cycle enzymes (the arginine synthetase system) diminishes during pregnancy (MACKIE and CAMPBELL, 1972). These authors demonstrated an increase ($\times 10$) in the activity of fructose 1-6 diphosphatase during the last two weeks of pregnancy, this could be the cause of the increase in glycemia at the end of pregnancy which we have observed. The increase in glucocorticoids which precede birth mainly due to foetal secretion (HARRISON *et al.*, 1972) provides an alternative explanation since this is known to have a considerable effect on neoglucogenesis from amino acids (REILLY and FORD, 1974).

Hypoalaninemia at the end of pregnancy indicates that ewes should receive in their diet a sufficient level of nitrogen as well as of energy. The level of alanine in the plasma can provide a good index of this, particularly since its enzymatic measurement is very simple. Alanine appears to be involved in regulation of gluconeogenesis (FRIEDRICH and SCHONER, 1974) and may thus have an antiketogenic action in sheep (unpublished results) comparable to that in man (GENUTH and CASTRO, 1974).

Neoglucogenesis from amino acids requires considerable energy, both for neoglucogenesis itself, and for ureogenesis, which includes necessarily the energy of compartmentalisation between the cytoplasm and mitochondria (BERRY, 1974).

Increased heat production at the end of pregnancy results in part from these reactions.

STEEL and LENG (1973) noted that increased neoglucogenesis is not necessarily associated with an increase in ketogenesis. KREBS *et al.* (1974) suggested that the increased energy utilization due to ureogenesis and neoglucogenesis may lead to more complete combustion of fatty acids if their supply remains constant. In fact, the limiting factor in ketogenesis is the quantity of fatty acids available.

Regulation of ketogenesis and acetate metabolism

Ketogenesis can be dissociated from neoglucogenesis, but the way in which this occurs is not completely clear. The first possibility may involve regulation of the level of FFA uptake by the liver for this increases following an increase in the ratio FFA/plasma albumin (SOLER-ARGILAGA, INFANTE and POLONOVSKI, 1973). In addition, the orientation to esterification of FFA can influence the level of β oxidation. Esterification may be reduced in animals showing hypoglycemia and a lack of glucose precursors (HEIMBERG *et al.*, 1974), while lack of insulin (BASSETT and MADILL, 1974) probably defavorises triglyceride liberation as lipoproteins (NIKKILA, 1974.)

An alternative regulation may be provided by acyl CoA penetration in mitochondria by the acylcarnitine transferase system. This would be controlled by nutritional (inhibition by glucose precursors) and hormonal factors (inhibition by insulin, activation by glucagon), and would constitute a limiting step in the orientation to β oxidation (MC GARRY, WRIGHT and FOSTER, 1975).

The control of β oxidation is not well known at present. It probably depends on the energetic state of the cell, controlled by the ratio NADH/NAD and FADH₂/FAD. Propionate has a very high antiketogenic activity affecting either β oxidation directly, the activity of tricarboxylic acids cycle or the enzymes of ketogenesis (BUSH and MILLIGAN, 1971). When the Krebs cycle is active ketogenesis is not proportional to the energy outlay : the fact that ketogenesis becomes of importance only when the liver cell lacks glucose precursors suggests that in these circumstances there is reduced activity of the Krebs cycle and considerable energy consumption. The problem is to determine the nature of this consumption since neoglucogenesis is then limited by lack of substrates (G₂ ewes of the HL and LL groups at the end of pregnancy). It seems quite remarkable that the increased heat loss during pregnancy is greatest in animals on a restricted diet (MCGRAHAM, 1968).

In addition to its use in ketogenesis, acetyl CoA may undergo deacylation to endogenous acetate (G₂ ewes of HL and LL groups). PALMQUIST (1972) showed that acetate could be produced from palmitate and that this production increased with fasting. Of the increase in plasma acetate, it is not possible to determine the relative importance of endogenous acetate and that resulting from an reduced metabolisation due to a lack of glucose (REID, 1968). Normally acetate is not ketogenic, but when there is a glucose deficit it may enrich the acetyl CoA pool of the liver cell and thus favorise ketogenesis.

RÉSUMÉ

VARIATIONS DE QUELQUES PARAMÈTRES PLASMATIQUES
DE LA NÉOGLUCOGENÈSE ET DE LA CÉTOGENÈSE CHEZ LA BREBIS GESTANTE
EN FONCTION DU PLAN DE NUTRITION

Quatre lots de huit brebis *Charmoises* pesant 50 à 60 kg ont été soumis au plan d'alimentation suivant à partir de la 9^e semaine de gestation :

- lot haut-bas (HL) : niveau de nutrition élevé jusqu'à la 15^e semaine (foin *ad libitum* + 0,8 kg de concentré) puis bas jusqu'à la mise bas (0,65 kg de foin),
- lot bas-haut (LH) : niveau bas puis élevé,
- lot bas (LL) : niveau bas durant la gestation (0,65 kg de foin puis 0,8 kg),
- lot témoin (C) : niveau alimentaire croissant durant la gestation (foin *ad libitum* + concentré 0,3 kg puis 0,5 kg).

Les concentrations plasmatiques en glucose, alanine et urée ont été mesurées pour préciser l'importance de précurseurs glucogéniques tels que l'alanine. Les liaisons entre le métabolisme lipidique et celui de l'acétate ont été étudiées en déterminant les taux plasmatiques d'acides gras libres (AGL), de 3-hydroxybutyrate et d'acétate.

Aucune brebis n'a présenté d'élévation importante des concentrations en 3-hydroxybutyrate ni d'hypoglycémie sévère dans les lots C et LH. Par contre, dans les lots HL et LL, les brebis gémeuses ont présenté durant le dernier mois de gestation tous les signes biochimiques de la toxémie de gestation, mais pas les signes cliniques. Durant toutes les périodes de sous-nutrition les taux d'alanine diminuent fortement, ce qui traduit une carence en substrats glucoformateurs conduisant à des hypoglycémies. Cependant, lorsque les glycémies restent élevées (lot HL, 1^{re} période), ces différences de glycémie entre brebis porteuses de simples ou de doubles ne se retrouvent pas au niveau de l'alanine.

La céto-genèse n'est importante que si le taux des AGL est élevé et si la glycémie et les taux de composés glucoformateurs sont faibles. C'est donc la différence de drainage du glucose et d'utilisation des composés glucoformateurs, autant que celle de la lipomobilisation, qui distingue les brebis porteuses d'un ou deux fœtus.

Le métabolisme des AGL captés par le foie semble étroitement dépendant de la néogluco-genèse : au niveau de la réestérification, de la pénétration dans la mitochondrie (inhibition du système de transfert carnitine acyl transférase par les composés glucoformateurs) et probablement de l'intensité de la β oxydation et de l'activité du cycle de Krebs.

Les taux d'acétate augmentent parallèlement à ceux de 3-hydroxybutyrate et d'AGL. Il y a vraisemblablement une augmentation de la production hépatique d'acétate endogène à partir de l'acétyl CoA, d'autre part les hypoglycémies peuvent conduire à une difficulté d'utilisation de l'acétate et du 3-hydroxybutyrate à la périphérie.

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