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GLUCAGON, INSULIN AND GLUCOSE HOMEOSTASIS IN THE FETAL LAMB

J.M. BASSETT

*Nuffield Institute for Medical Research, University of Oxford,
Headley Way, Headington, Oxford OX3 9DS, England*

Résumé

HOMEOSTASIE DU GLUCAGON, DE L'INSULINE ET DU GLUCOSE CHEZ LE FŒTUS D'AGNEAU. — Les actions de l'insuline et du glucagon chez le fœtus d'agneau, et la régulation de leur sécrétion par le pancréas fœtal ont été étudiées afin d'envisager le rôle possible de ces hormones dans la régulation de l'homéostasie du glucose chez l'agneau pendant la vie fœtale.

Il y a de nombreux indices d'une stimulation de l'utilisation du glucose sous l'influence de l'insuline chez le fœtus d'agneau, et de la mobilisation du glycogène du foie fœtal sous l'effet du glucagon.

Le glucose stimule, et l'adrénaline inhibe la sécrétion d'insuline par des fragments de pancréas fœtal *in vitro* à partir du 50^e jour de la gestation, mais l'alanine et la glycine ont peu d'effet sur la libération d'insuline.

L'alanine et la glycine stimulent la sécrétion de glucagon par des fragments de pancréas fœtal *in vitro* à partir du 50^e jour de gestation. Ces effets sont potentialisés par la caféine. L'adrénaline a un faible effet stimulant, mais la libération de glucagon n'est pas altérée par le glucose. *In vivo*, une perfusion d'adrénaline accroît la concentration de glucagon plasmatique fœtal, ce que ne fait pas une perfusion de glycine. Une perfusion de glycine chez des agneaux nouveau-nés accroît le glucagon plasmatique. Un jeûne de deux jours chez la brebis gestante provoque une baisse de l'insuline plasmatique mais ne modifie pas le glucagon, aussi bien chez la mère que chez le fœtus.

Ces observations suggèrent que la sécrétion d'insuline chez l'agneau fœtal est un facteur important du captage et de l'utilisation du glucose chez le fœtus, du moins pendant le dernier tiers de la gestation. L'importance quantitative du glucagon dans la régulation du métabolisme du glucose hépatique chez le fœtus reste incertaine.

Homeostatic mechanisms are elegantly demonstrated in the regulation of blood glucose concentrations. With glucose the major substrate for oxidative metabolism in tissues of the nervous system, it is hardly

surprising that such a sophisticated system of integrated neural and endocrine controls should have evolved to ensure constancy in the supply of their principal fuel.

In normal man, despite large variations in

the input and output of glucose associated with meals, exercise and so on, blood glucose remains within a remarkably narrow range largely because of changes in the secretion of insulin and glucagon, the two principal hormones regulating minute by minute flux of glucose within the system (Molnar *et al.*, 1972; Unger, 1976). Similarly in non-pregnant sheep plasma glucose concentrations are maintained within a narrow range over a wide range of dietary nutrient intakes even though little glucose is derived directly from dietary sources. Virtually all glucose in sheep is derived via gluconeogenic pathways because of the nature of digestion in the rumen. Even so, there are large differences in the rate of glucose flux related to differences in digestible nutrient intake and it is evident that regulation of glucose metabolism by insulin and glucagon is just as important as in man (Bassett, 1975).

Pregnancy, however, provides a very severe challenge to maternal glucose homeostasis. In late pregnancy especially the rapidly increasing demands of the fetus for glucose make the maintenance of normal blood glucose levels in the mother difficult except under the most optimum dietary conditions. Furthermore, it is evident that the developing fetus, via the secretion into the maternal circulation of hormones produced in the placenta, can modify metabolism in maternal tissues to maximize delivery of glucose to the uterus. The consequences of undernutrition during late pregnancy in the sheep for maternal blood glucose homeostasis and metabolic regulation are now well known from the comprehensive studies of Reid and his associates (Reid, 1968). What is clearly evident is that fetal well being is preserved at the expense of maternal glucose homeostasis with the ultimate consequences of acute hypoglycemia and ketosis in clinical pregnancy toxemia if the undernutrition is not corrected. Nevertheless there is substantial evidence that fetal blood glucose concentrations are very closely correlated with maternal glucose concentrations in the sheep and other species which have been studied (for review see Silver, 1976) and it can be questioned whether the fetus has any independent control of its glucose homeostasis. However, while the need for homeostatic mechanisms may be less during fetal life because the infant is a least partially

protected by maternal homeostatic mechanisms from many of the vicissitudes of the environment, pre-natal development of these mechanisms would be prudent preparation for the dramatic changes occurring at birth; furthermore fetal development of glucose homeostatic mechanisms might be essential for optimal growth and development during this period particularly in species where gestation is prolonged.

Indeed the realization that glucose derived from the mother, though quantitatively important, was not the sole source of fuel for fetal oxidative metabolism (Battaglia and Meschia, 1973) has meant that it is necessary to consider more carefully whether glucose utilization by fetal tissues is regulated solely by its availability or whether fetal endocrine mechanisms may be important in determining the metabolic rate in fetal tissues of glucose derived from the mother, and also whether the fetus may be able to contribute to maintenance of its glucose requirements when maternal supplies are severely limited. Many endocrine mechanisms may be involved but the extent to which fetal glucose metabolism can be regulated will depend largely on the functional maturity of the glucagon and insulin secreting cells in the fetal pancreas and on the presence of metabolic pathways sensitive to the action of these hormones.

Development of Insulin and Glucagon Secretion

Insulin-secreting β cells and glucagon-secreting α cells of the pancreatic islets develop relatively early in gestation (Van Assche *et al.*, 1976). Beta cells can be detected by immunofluorescent techniques in the islets of fetal lambs at 40 to 50 days of pregnancy (Willes *et al.*, 1969) and both insulin and glucagon can be extracted with acid-ethanol from the pancreas in significant quantities from as early as 50 days gestation (Fig. 1). The presence of both hormones in fetal blood at concentrations in the same range as those in maternal blood during the latter two-thirds of pregnancy shows they are being released into the circulation as there is little evidence for transplacental flux of either hormone (Alexander *et al.*, 1972, 1973). It cannot be presumed, however, that the glucagon-like immunoreactivity (GLI) present in fetal plasma is neces-

sarily all of pancreatic origin unless a highly specific antibody has been used for its measurement. Determination of GLI by means of a "non specific" antibody demonstrates the presence in acid-ethanol extracts of fetal sheep gastro-intestinal tract tissue of significant amounts of enteroglucagon (Gut GLI). The highest concentrations are found in the terminal ileum and large intestine and the material is present from the earliest time studied; the amount present increasing with fetal development (Fig. 2). The material behaves on gel-filtration like entero-glucagon from other species (Valverde *et al.*, 1970) and is clearly distinct from pancreatic glucagon. Measurement of plasma GLI with the non-specific antibody used in these studies gave values in fetal plasma 3 fold or more greater than values obtained with an antibody (Unger, 30K) highly specific for pancreatic

glucagon. It is evident that substantial amounts of entero-glucagon are being secreted during fetal life and indeed its secretion can be stimulated by infusing glucose into the gut of the fetal lamb *in utero* (Madill and Bassett, unpublished observation) or in acute studies (Alexander *et al.*, 1977), but the physiological role of this presumptive gastrointestinal hormone remains to be elucidated. Its presence clearly makes the interpretation of plasma glucagon values obtained with non-specific antibodies uncertain. Nevertheless it appears that glucagon and insulin are both being actively secreted in the fetal lamb during the latter two-thirds of gestation, so are potentially able to take part in active fetal metabolic regulation, provided (a) that the necessary "hormone-sensitive" metabolic pathways are present in the fetal tissues and (b) that the secretion of insulin and glucagon from the fetal pancreas can be regulated.

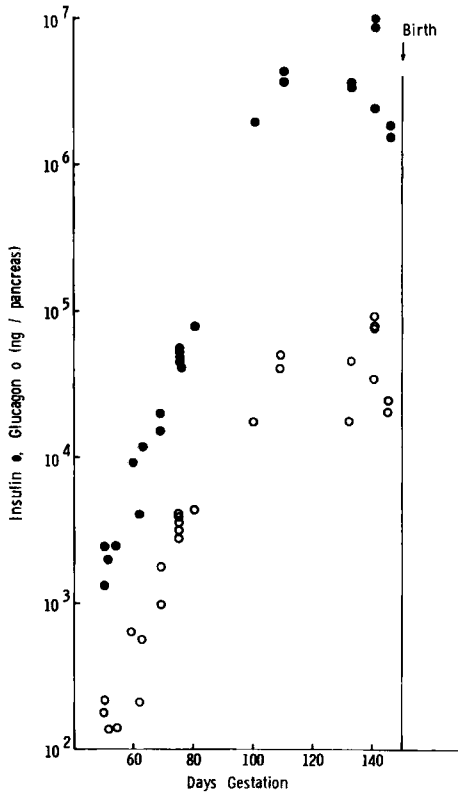


Fig. 1: Insulin (●) and glucagon (○) content (ng/pancreas) in pancreas tissue from fetal sheep.

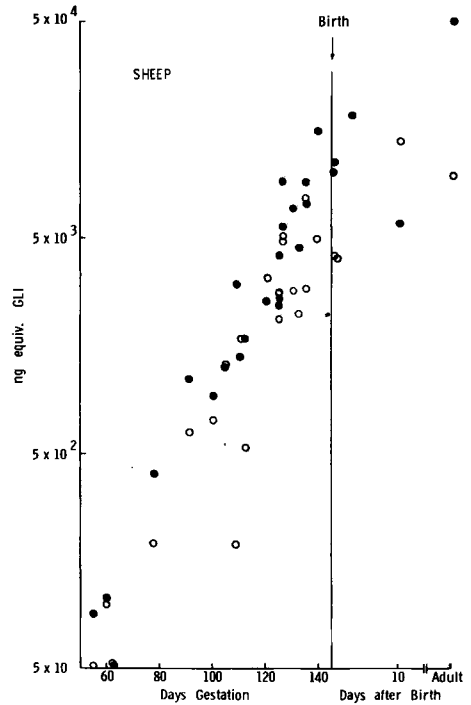


Fig. 2: Immunoreactive glucagon (GLI) content (ng equiv./tissue) in the terminal ileum (distal 30% by weight small intestine) (●) and the large intestine (○) of fetal sheep.

Insulin and Glucagon regulation of metabolism in the fetal lamb

a) *Insulin*

Although the plasma glucose concentration of the fetal lamb (0.5 — 1.5 mM) is much lower than that of postnatal lambs and is maintained by flux of glucose across the placenta, infusion of insulin into chronically cannulated fetuses during the last third of pregnancy decreases their plasma glucose (Shelley, 1973) and increases their rate of glucose utilization as determined from umbilical arterio-venous differences (Colwill *et al.*, 1970) or measurements of umbilical uptake (Simmons *et al.*, 1975). Similarly the destruction of fetal β cells by streptozotocin results in increased plasma glucose and impaired removal of infused glucose (Shelley, 1975). However, the fetal tissues and metabolic steps involved in the insulin stimulation of fetal glucose utilization in the lamb remain to be determined. The specific binding of (¹²⁵I) insulin to partially purified membranes from liver, heart and skeletal muscle tissue of fetal lambs (90-145 days gestation) (Michael and Bassett, unpublished observations) indicates the presence of receptor mechanisms for insulin, but the effects of insulin on glucose metabolism have not been determined. Insulin stimulates the metabolism of glucose in muscle of fetal monkeys (Boceck *et al.*, 1973) and incorporation of glucose into glycogen in fetal rat liver (Eisen *et al.*, 1973). While many uncertainties remain, it is evident that insulin can play a significant role in regulating fetal glucose metabolism. Furthermore many observations linking fetal insulin status with fetal growth (Liggins, 1976) suggest important effects of this hormone on fetal amino acid metabolism and give added relevance to studies of the mechanisms regulating insulin secretion in fetal life.

b) *Glucagon*

Infusion of glucagon into fetal lambs during the last third of gestation results in significant increases in their plasma glucose concentration (Bassett and Thorburn, 1971; Warnes *et al.*, 1977; Alexander *et al.*, 1976). The effect is probably due entirely to stimulation of fetal hepatic glycogenolysis since

the necessary enzymes (Ballard and Oliver, 1965) and specific glucagon receptors (Michael and Bassett, unpublished observations) are present in the liver of the fetal lamb throughout the last third of gestation. All the enzymes necessary for gluconeogenesis are also present in fetal lamb liver during this period, but studies on the incorporation of lactate into glucose in chronically cannulated fetal lambs (Warnes *et al.*, 1977) suggest that no significant gluconeogenic activity is present before birth. Thus it would appear that while the fetal liver under the influence of glucagon may be able to contribute in the short-term to fetal glucose regulation through mobilization of glycogen it seems questionable whether gluconeogenesis, regulated by glucagon, plays any quantitatively significant part in fetal glucose homeostasis, although such a mechanism would have seemed a possibility in view of the large increase in amino acid catabolism observed during a prolonged fast (Simmons *et al.*, 1974).

It would appear therefore that the necessary mechanisms exist to allow both insulin and glucagon to participate in the regulation of fetal metabolism during the last third of gestation in the sheep, the period when quantitatively the biggest part of fetal growth occurs and maternal nutritional status has its greatest effects on size at birth (Wallace, 1948).

The regulation of fetal insulin and glucagon secretion

a) *Insulin*

From the studies of Shelley (1973) and Bassett and Madiil (1974a) there can be little question that the plasma insulin concentration of the fetal lamb in the last third of gestation, like that of the ewe herself, is related to the nutritional status of the ewe, but it is not evident that there are changes associated with increasing fetal age.

As it seems unlikely that variations in the secretion of the gastro-intestinal hormones play any major role in regulating insulin secretion *in utero*, fetal insulin secretion is likely to depend principally on regulation by circulating metabolites and on autonomic nervous system function. On the other hand, gastro-intestinal regulation of insulin release

cannot be ignored entirely since large quantities of amniotic fluid are swallowed by fetuses in later gestation (Bradley and Mistretta, 1973).

In post-natal life glucose is unquestionably the most important circulating metabolite involved in regulating insulin secretion but studies on several species have questioned its importance as a regulator of insulin secretion during fetal life. Early studies of glucose action on insulin release in the fetal lamb suggested it was a poor stimulus. However, subsequent studies have shown that glucose can stimulate insulin secretion from the pancreas of the fetal lamb, both *in vivo* and *in vitro*. The kinetics for glucose stimulation of insulin secretion from the fetal pancreas differ substantially from those for glucose action on post-natal pancreas (Bassett *et al.*, 1973). There is little evidence *in vivo* or *in vitro* for any rapid insulin secretory response, but small increases in glucose within the physiological concentration range of 0.5 to 2 mM stimulate an increase in insulin concentration *in vivo* (Shelley, 1973; Bassett and Madill, 1974b; Fiser *et al.*, 1974a) and can increase insulin secretion *in vitro* (Bassett *et al.*, 1973.) Indeed, *in vitro* studies indicate that glucose stimulation of insulin secretion from fetal lamb pancreas is maximal at concentrations around 5 mM in contrast to the situation with post-natal pancreas where 5 mM is little above the threshold for stimulation of insulin secretion (Bassett *et al.*, 1973) although the

reason for these different kinetics remains unexplained. However, the results of these studies of glucose action *in vivo* and *in vitro* are good reason to suppose that the close correlation between glucose and insulin levels of fetal lambs during nutritional studies, though markedly different from the relation in the ewe (Fig. 3), reflects a causal dependence of insulin secretion on glucose concentration in this situation and implies that physiological variation in fetal glucose is an important determinant of insulin secretion and thereby of glucose utilization in insulin sensitive tissues. The absence of any acute insulin response to glucose seems of little relevance in the regulation of fetal glucose utilization. While there is strong evidence for this view from studies carried out during the last third of gestation little is known about how early this mechanism becomes significant in fetal metabolic regulation. *In vivo* studies of fetal pancreas function earlier than 90 days gestation present considerable problems but fetal lamb pancreas tissue can be studied *in vitro* from a very early stage. Using a continuous-flow (perfusion) incubation technique (Bassett and Madill, unpublished) it has been possible to show that 6 mM glucose stimulates insulin secretion using pancreas pieces from a 50 day fetus (Fig. 4). With pancreas tissue from fetuses 50-100 days gestation little glucose stimulates insulin secretion more consistently in the absence of caffeine than it does with older fetal pancreas tissue.

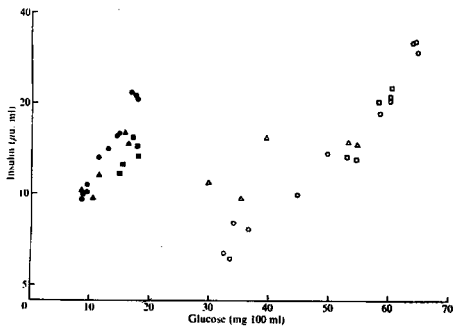


Fig. 3: Relationship between mean plasma glucose and insulin concentrations in ewes (open symbols) and their fetuses (closed symbols) during a 24 h feeding cycle and a 48 h fast (\circ), during restricted feeding (\square) and during *ad libitum* feeding (\triangle) (from Bassett and Madill 1974a with kind permission of the Editor of the Journal of Endocrinology).

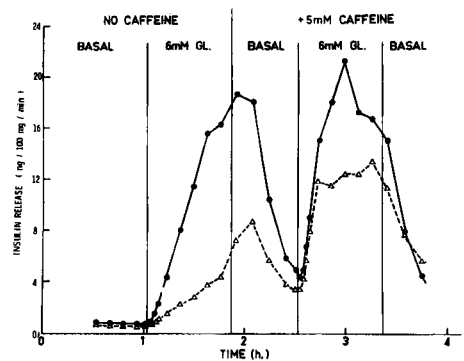


Fig. 4: Insulin release by pancreas pieces from 2 fetal lambs at 50 (\bullet) and 69 (\circ) days gestation. Tissue was perfused *in vitro* with a bicarbonate medium. 6 mM glucose and 5 mM caffeine were added at the times indicated. Each line is the mean of 4 separate perfusions.

Nonetheless 5 mM caffeine potentiates the response at all stages, though the physiological relevance of this *in vitro* finding is far from clear. It is evident therefore that the circulating glucose concentration is potentially able to regulate insulin secretion in the fetal lamb—albeit through relatively slow changes in the secretion rate—throughout two-thirds of gestation.

In other species, because of the “obtunded” response of insulin secretion in response to glucose, attention has been focused on the amino acids as possible alternative regulators of insulin secretion. *In vitro* studies with pancreas tissue from several species suggest a number of amino acids such as leucine and arginine may be more effective stimuli than glucose, but, although fetal amino acid levels may be substantially higher than maternal concentrations there is no evidence whether physiological alterations in their concentration *in vivo* play any part in modulating insulin secretion during fetal life. Brief (10 min.) infusions of alanine into fetal lambs *in utero* (Fiser *et al.*, 1974a) did not significantly increase plasma insulin although similar infusions in newborn lambs did do so (Fiser *et al.*, 1974b). Studies *in vitro* (Bassett, unpublished) with a range of amino acids including alanine, glycine, serine and leucine at a concentration of 5 or 10 mM have indicated that only leucine has any significant stimulatory effect on insulin secretion from fetal lamb pancreas tissue. However, it must remain questionable whether leucine at normal physiological concentrations plays any significant role in regulating insulin secretion.

Despite the low circulating glucose concentration it appears, therefore, that glucose itself is the principal metabolic regulator of insulin release prior to birth in the lamb.

The significance of neural regulatory mechanisms remains relatively unexplored. Shelley (1975) has observed that hypoxia inhibits the insulin-secretory response to hyperglycemia probably via catecholamine release in this situation (Jones and Robinson, 1974). Adrenaline administration to chronically cannulated fetal lambs near term inhibits insulin secretion (Jones and Ritchie, 1977; Bassett, Jones and Walker, unpublished observations) just as in adult sheep (Bassett, 1970) or young lambs (Bassett and Alexander, 1971) and *in vitro* observations (Bassett, unpublished) show that adrenaline

is a potent inhibitor of glucose-stimulated insulin secretion from fetal lamb pancreas at the earliest stage examined (55 days gestation). Insulin secretion can thus be modulated by adrenergic inhibitory mechanisms throughout fetal life, but the extent to which this may be brought about by circulating catecholamines or more directly by the sympathetic innervation of the pancreas has not been investigated. Stimulatory vagal effects on insulin release from the fetal pancreas also do not appear to have been sought. However, acetylcholine causes a marked stimulation of insulin secretion from perfused fetal lamb pancreas pieces in the presence of glucose and caffeine (Bassett, unpublished observations) so cholinergic mechanisms appear potentially able to participate in regulation of insulin secretion in fetal life.

Although the role of glucose as a regulator of insulin secretion now seems well established, despite the relatively slow response of the β cell, the possible significance of any of the alternative β cell regulatory mechanisms remains uncertain. It is evident, however, that adrenergic inhibitory mechanisms may be of significance in pathological states such as hypoxia but tonic regulation of basal insulin release through adrenergic and cholinergic mechanisms which might be of greater long-term significance for fetal glucose homeostasis has not been investigated.

b) Glucagon

Information about the plasma concentration of glucagon and its regulation in fetal lambs is far more limited than is the case for insulin. This is very largely a consequence of the difficulties involved in measurement of pancreatic glucagon. Most of the values reported have been obtained using antisera cross-reacting at least to some extent with enteroglucagon and as indicated earlier the presence of this material cannot be ignored.

Published observations indicate that the regulation of glucagon secretion from the fetal pancreas may differ from that of the adult. Fiser *et al.* (1974a), Shelley *et al.* (1975) and Alexander *et al.* (1976) have reported that plasma glucagon concentrations in the fetal lamb, either chronically cannulated *in utero* or exteriorized under anaesthe-

sia during acute studies, are not significantly influenced either by hyperglycemia or by hypoglycemia. A similar lack for response to change in glucose concentration has been observed in rat (Girard *et al.*, 1974) or monkey fetuses (Chez *et al.*, 1974). Consistent with these observations and with findings on adult sheep we have found that plasma glucagon concentrations, determined with an antibody specific for pancreatic glucagon (Unger, 30K), did not change significantly during a 24 h feeding cycle and a subsequent 48 h fast in either mother or fetus in two twin-pregnant animals with one fetus chronically cannulated (Fig. 5). Judging from plasma glucose and insulin concentrations both ewes were relatively undernourished although offered food *ad libitum*. Despite this their plasma glucagon concentrations and those of the cannulated fetuses were comparable to those of other animals in our studies and to basal values, determined using an antibody specific for pancreatic glucagon, in

non-pregnant sheep (Bassett, 1972; Arcus *et al.*, 1976). Plasma glucagon concentrations of non-pregnant adult sheep also do not change during a four day fast and change little during hyperglycemia (Bassett, 1972) although acute insulin-induced hypoglycemia does result in a marked increase in plasma glucagon concentration (Arcus *et al.*, 1976; Alexander *et al.*, 1976), due presumably to direct sympathetic stimulation of pancreatic glucagon secretion rather than to direct effects of hypoglycemia since a similar effect was not seen in pancreas tissue transplanted to the neck of the same animals (Arcus *et al.*, 1976). These observations all suggest that changes in glycemia in sheep may be relatively unimportant in influencing glucagon secretion directly, although clearly central effects of acute hypoglycemia mediated either directly via sympathetic stimulation of the α cells or indirectly via increased circulating catecholamine concentrations have an important role. However it should be pointed out that irrespective of whether or not glucagon concentrations increase during fasting, diminished insulin concentrations will lead to a fall in the plasma insulin/glucagon ratio and increased dominance of hepatic metabolism by the effects of glucagon (Unger, 1971) and this may be just as relevant in the fetus as in the adult.

Whereas there is only equivocal evidence for direct effects of plasma glucose concentration on glucagon secretion in either fetal or adult sheep there is stronger evidence that glucagon secretion in this species can be influenced by amino acids and the catecholamines, the principal glucagon secretagogues in most species (Unger and Orci, 1976).

In adult sheep and lambs short infusions of alanine result in significant increases in plasma glucagon (Fiser *et al.*, 1974a; Fiser *et al.*, 1974b). Significant increases in glucagon secretion have also been observed in adult sheep infused with arginine (Bryce *et al.*, 1975; Arcus *et al.*, 1976). Similarly intravenous or abomasal infusion of an amino acid mixture into growing lambs or adult sheep increases plasma GLI probably by increasing pancreatic glucagon secretion (Madill and Bassett, unpublished observation 1977). In fetal and newborn lambs alanine is evidently ineffective in stimulating glucagon release (Fiser *et al.*, 1974a; 1974b) although a response can be obtained in the newborn by concurrent infusion of theophyl-

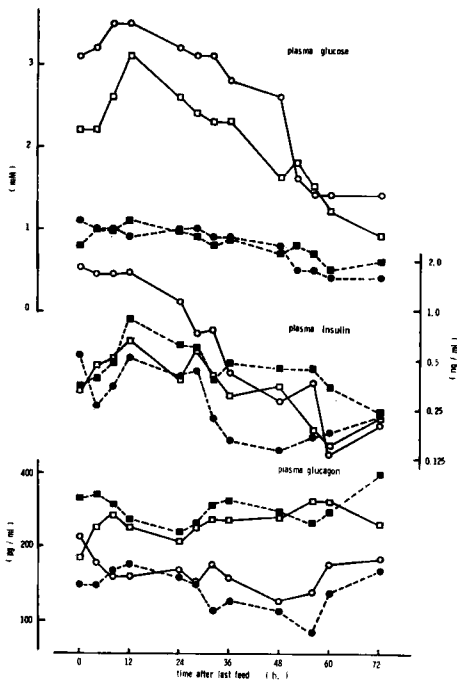


Fig. 5: Changes in plasma glucose, insulin and glucagon concentrations in 2 twin pregnant ewes (○□) and one of their fetuses (●■) during a 24 h feeding cycle and a subsequent 48 h fast. E 1-118 days pregnant; E 22-130 days pregnant.

line (Fiser *et al.*, 1976) and immaturity of glucagon secretion mechanisms in the fetal α cell has been suggested. However arginine infusions into exteriorized fetuses (Alexander *et al.*, 1976) which caused large rises in their plasma α amino nitrogen concentrations resulted in significant increases in glucagon secretion.

Since most of these studies involved amino acid infusion rates far in excess of those likely to occur *in vivo* the physiological relevance of the findings remains questionable. Accordingly we have attempted to assess a) whether, and how early, in fetal life glucagon secretion from the pancreas of the lamb can be stimulated *in vitro* by a variety of amino acids and b) whether slow intravenous infusions of amino acids at rates closer to usual entry rates can be shown to alter plasma glucagon concentrations *in vivo*.

Although glycine has received little attention as a regulator of glucagon secretion it is quantitatively the most important amino acid in sheep plasma both before (Hopkins *et al.*, 1971; Schulman *et al.*, 1975) and after birth (Hogan *et al.*, 1968) and its concentration in the adult is inversely

related to the nutritional state. Further it is among the most potent amino acids stimulating glucagon secretion in the dog (Marreiro Rocha *et al.*, 1972). In our studies we therefore sought to determine whether glycine might be a physiologically important regulator of glucagon release in the lamb.

In studies *in vitro* with perfused lamb pancreas pieces 5 mM glycine and alanine are consistently the most potent amino acids stimulating glucagon release. Several other amino acids including arginine stimulate glucagon release to a lesser extent, but leucine is ineffective (Fig. 6). Consistent with *in vivo* observations, glycine or alanine-stimulated glucagon secretion from perfused pancreas pieces is not influenced by the inclusion of 20 mM glucose in the incubation medium. Glycine and alanine likewise readily stimulate glucagon secretion from pancreas tissue of fetal lambs, responses to both having been observed with tissue from the youngest fetuses studied. While the presence of caffeine potentiates amino acid-stimulated glucagon release stimulatory effects can be obtained in its absence (Fig. 7). *In vivo*, continuous slow intra-venous infusion of glycine or alanine at a rate of 1 mM/kg/h for two hours caused a steady

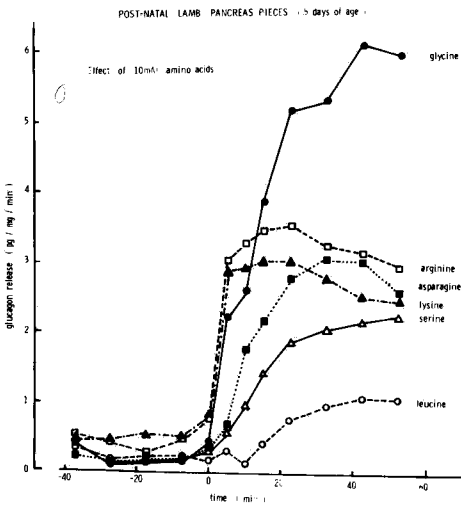


Fig. 6: Glucagon release by pancreas pieces from a lamb 5 days of age. Tissue was perfused *in vitro* with a bicarbonate medium containing caffeine, pyruvate, fumarate and glutamate, each at 5 mM. Amino acids (10 mM) were added after an 80 minute control period. Each line is the mean of two incubations.

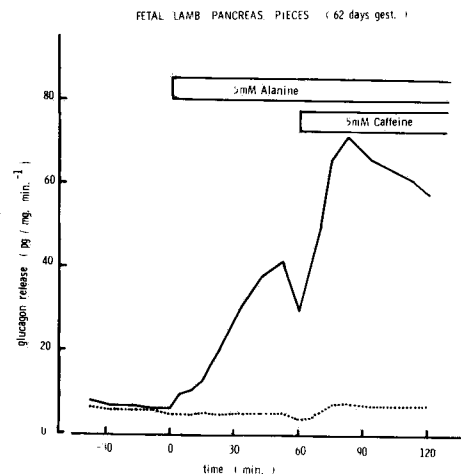


Fig. 7: Glucagon release *in vitro* by pancreas pieces from a fetal lamb at 62 days gestation. The conditions of incubation were the same as in Fig. 6, except that caffeine (5 mM) was added 1 h after alanine (5 mM). Each line is the mean of 4 separate perfusions.

increase in the plasma glucagon concentration of 4 to 8 week old lambs. In lambs less than two weeks old, glycine or alanine infusion at similar rates failed to cause any consistent stimulation of glucagon release, but infusions at a higher rate were effective. Infusion of glycine at 1 mM/kg/h (approx.) for two hours into 5 chronically cannulated fetal lambs also failed to cause significant stimulation of glucagon release. However although these observations are consistent with the negative findings of Fiser *et al.* (1974), the findings of Alexander *et al.* (1976) with arginine and the results of our *in vitro* studies suggest that immaturity of secretory mechanisms in the fetal α cell is an unlikely explanation for the *in vivo* observations. The ineffectiveness of individual amino acids infused *in vivo* may relate to the very high concentration of amino acids in fetal blood compared with those of the post-natal and adult animal but whether because basal amino acid concentrations are maximally stimulating the α cells or because the α cells are relatively insensitive to amino acid stimulation cannot be determined. Certainly the possibility exists that the high amino acid concentrations of the fetal lamb may provide a tonic stimulus to glucagon secretion. However, in view of the paucity of information about physiological variation in plasma amino acid concentrations it is impossible to know how significant such an action might be. It is of interest in this respect that Alexander *et al.* (1976) observed a significant correlation between initial fetal glucagon and amino nitrogen concentrations in their study. On the other hand fetal plasma glucagon concentrations are frequently lower than maternal concentrations despite the higher fetal amino acid concentrations (Shelley *et al.*, 1975; Bassett, unpublished observations) so it seems more likely that fetal pancreatic α cells are relatively insensitive to stimulation by amino acids.

Adrenaline infusion in the adult sheep increases plasma glucagon markedly by an α adrenergic receptor mechanism (Bassett, 1972). Comparable observations on fetal or newborn lambs, do not appear to have been reported. However, glucagon secretion is stimulated by adrenaline in fetal rats (Girard *et al.*, 1974) and by direct autonomic nervous system action in calves (Bloom *et al.*, 1973), consistent with observations on adults.

In recent studies in our laboratory (Bassett, Jones and Walker, unpublished observations) intravenous infusion of adrenaline at a rate of 1.3 $\mu\text{g}/\text{min}$. into chronically cannulated fetal lambs 120-144 days gestation significantly stimulates glucagon secretion (Fig. 8) confirming that functional adrenergic stimulatory mechanisms are present in the pancreatic α cell of the fetal lamb. Such adrenergic stimulation of glucagon release may be the mechanism by which hypoxia results in increased glucagon release in the fetus (Shelley *et al.*, 1975). Stimulatory effects of adrenaline on glucagon release have also been observed *in vitro* in perfusion studies on fetal pancreas tissue, though this effect has not been obtained so consistently as has inhibition of insulin secretion. Despite this limitation some stimulation of glucagon release by adrenaline has been observed *in vitro* with pancreas tissue from 55 and 62 day fetal lambs as well as from older fetuses, indicating that adrenaline is potentially able to stimulate glucagon secretion during much of fetal life in the lamb.

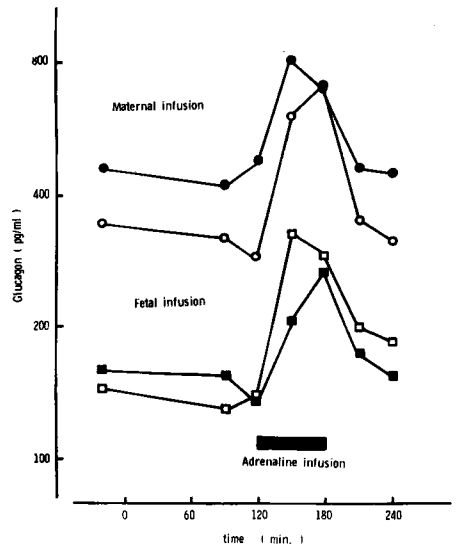


Fig. 8: Plasma glucagon concentrations in a pregnant ewe (\circ ●) during two separate i.v. infusions of adrenaline (13.5 $\mu\text{g}/\text{min}$. for 60 min.) and in her fetus on two other occasions at 120 and 122 days gestation (Δ ▲) when adrenaline (1.3 $\mu\text{g}/\text{min}$. for 60 min.) was infused into the fetus (Bassett, Jones and Walker, unpublished observations).

General discussion

The observations on plasma insulin levels in fetal lambs in a variety of situations all clearly indicate insulin plays an important role in regulating fetal glucose utilization throughout the latter third to half of gestation. It is evident that despite the absence of any rapid insulin secretory response to glucose, changes in the fetal plasma glucose concentration itself are the main determinant of changes in insulin secretion. Furthermore it seems very likely, although absence of information about insulin action in the fetus makes it impossible to be certain, that the reduced fetal insulin concentrations seen during maternal fasting play an important part in the fetal metabolic adaptation to reduced glucose availability (Simmons *et al.*, 1974). However it remains very uncertain what role glucagon may play. Relative dominance of fetal liver metabolism by the actions of glucagon may lead to glycolysis and a net outflow of glucose from the liver in this situation, but in the absence of evidence for significant rates of gluconeogenesis during fetal life it is difficult to believe that this can be of much quantitative significance during prolonged undernourishment.

Since the delivery of glucose to the fetus is largely dependent on the size of the maternal fetal gradient across the placenta (see review by Bassett and Jones, 1976) it is evident that fetal insulin by promoting glucose uptake in fetal tissues plays an important part in amplifying the transfer of glucose to the fetus and thereby may play an important role in determining fetal growth. Maintenance of normal glucose concentrations in the maternal supply to the placenta is therefore of paramount importance if fetal insulin secretion is to be promoted and the potential for fetal growth is to be realized. At present we still know little about the precise actions of insulin on glucose and protein metabolism in fetal tissues but it is clearly evident that if fetal growth is to be optimized maintenance of glucose homeostasis in both mother and fetus is essential.

Acknowledgements

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Summary

The actions of insulin and glucagon in the fetal lamb and regulation of their secretion from the fetal pancreas have been examined to assess the possible roles of these hormones in regulating glucose homeostasis in the lamb during fetal life. Much evidence indicates that insulin stimulates glucose utilization in the fetal lamb and that glucagon can promote mobilization of fetal liver glycogen. Glucose stimulates and adrenaline inhibits insulin secretion by fetal pancreas pieces *in vitro* from 50 days gestation onwards, but alanine and glycine have little effect on insulin release. Alanine and glycine stimulate glucagon secretion by fetal pancreas pieces *in vitro* from 50 days gestation. The effects are potentiated by caffeine. Adrenaline has a small stimulatory effect but glucagon release is not altered by glucose. *In vivo* adrenaline infusion increases fetal plasma glucagon concentrations but glycine infusion does not. Glycine infusion into post-natal lambs increases plasma glucagon. Fasting pregnant ewes for two days decreases plasma insulin but does not alter plasma glucagon in either ewe or fetus. The observations suggest insulin secretion in the fetal lamb is an important determinant of glucose uptake and utilization by the fetus during at least the last third of pregnancy. The quantitative importance of glucagon in regulating fetal hepatic glucose metabolism remains uncertain.

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