Plasma glucagon levels in the term human infant and effect of hypoxia

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Johnston, D. I., and Bloom, S. R. (1973). *Archives of Disease in Childhood*, 48, 451. Plasma glucagon levels in the term human infant and effect of hypoxia. The mean plasma pancreatic glucagon level at birth in 44 normal infants delivered vaginally was 140 pg/ml. The simultaneous maternal level was 122 pg/ml which was not quite significantly different at the $P < 0.05$ level. 2 hours after birth 30 of these infants had a mean rise in plasma glucagon of 51 pg/ml ($P > 0.01$), and their mothers had a fall of 38 pg/ml ($P > 0.05$). The mean plasma pancreatic glucagon level at birth in 12 normal infants delivered by caesarean section was 130 pg/ml which did not differ significantly from the group delivered vaginally. The glucagon level at birth in 20 infants with fetal distress (fetal scalp pH < 7.2 or umbilical artery pH < 7.15) was 244 pg/ml, and this was significantly higher than for normal infants at birth ($P > 0.01$). Whereas the rise in neonatal glucagon 2 hours after birth might have been caused by a mean fall in blood glucose of 23 mg/100 ml, the infants with fetal distress had normal glucose levels, so that another mechanism must be responsible for their raised glucagon.

The level of pancreatic glucagon at birth and the change that occurs in the first hours of life have not previously been reported. The adaptation of the human fetus to an independent existence requires mobilization of energy stores. The fetus accumulates a considerable reserve of hepatic glycogen in the latter part of gestation (Shelley, 1961) and this appears to be responsible for the maintenance of blood glucose after birth (Shelley and Neligan, 1966). Glucagon has been shown to activate hepatic glycogenolysis and gluconeogenesis (Park and Exton, 1972) and may play a significant role in energy homeostasis of the newborn. Studies in the rat indicate that it may also induce the synthesis of certain rate-limiting enzymes in the gluconeogenic pathway (Philippidis and Ballard, 1970).

Liver glycogen is rapidly depleted when animals are exposed to acute anoxia (Mott, 1961). In the human it is not possible to measure hepatic glycogenolysis directly but the hypoxic fetus appears to raise its plasma glucose and lactate (Stembera and Hodr, 1966; Gennser and Nilsson, 1971), and it may be inferred that this is the result of the mobilization of liver glycogen.

The two difficulties that have hitherto impeded the radioimmunoassay of human neonatal pancreatic glucagon are lack of sensitivity and cross-reaction with enteroglucagon, an immunologically similar hormone released from the small intestine. The raising of a highly avid and relatively specific antibody has sufficiently overcome these problems to permit glucagon assay even with the small plasma volumes which it is reasonable to obtain in human neonatal research.

Patients and methods

Fifty-six mothers and infants who had Apgar scores of 7 or above at 1 minute were studied. 44 were delivered vaginally and 12 by elective caesarian section, performed either because of pelvic disproportion or because of a previous caesarean section; there was no indication of fetal distress in this group. The majority of the mothers received intravenous infusions of glucose during labour. The mean infant birthweight was 3.4 kg (range 2.75 to 3.88) and all were appropriate for gestational age (Tanner and Thomson, 1970).

In a separate group of 20 infants labour was complicated by clinical signs of fetal distress—irregularities of the fetal heart or release of meconium. The management of these cases followed the scheme described by Beard *et al.* (1971) in which the fetal condition was monitored by fetal scalp pH measurement and conti-

nuous fetal heart rate recording. In all these 20 cases fetal acidosis was shown (fetal scalp pH <7.20 and/or umbilical artery pH <7.15) and was an indication for forceps extraction in 8, and emergency caesarean section in 12. 17 of the infants had an Apgar score of less than 7 at 1 minute. The mean birthweight (range) of this group was 3.0 kg (2.19 to 3.71) and 3 were below the tenth centile for their gestational age.

Blood samples were collected at the time of delivery from a maternal antecubital vein, and from the umbilical vein and artery. The latter were obtained from a segment of the umbilical cord which had been isolated by double clamps at the earliest opportunity.

Two hours after delivery a further blood sample was taken from 30 of the normal infants who had had a vaginal delivery and also from their mothers. The infant blood sample was taken from a peripheral vein (usually an antecubital vein) and preceded the first feed. Permission was obtained from the mother to perform the venepuncture.

The blood for glucagon assay (1 ml) was immediately placed in a chilled heparinized tube containing 1000 KIU (kallikrein inhibitor units) aprotinin. The plasma was separated and frozen within 15 minutes of collection. True glucose was measured by a glucose oxidase method after deproteinization (Marks, 1959).

An antiserum was raised in a New Zealand white rabbit to Novo porcine pancreatic glucagon coupled to serum albumin by carbodi-imide condensation (Assan *et al.*, 1965). The initial and boosting doses contained 200 µg of glucagon each and were given at 3-monthly intervals by multiple subcutaneous injections in complete Freund adjuvant. Maximum avidity and titre were reached after 1 year. The antiserum was found to react strongly with pancreatic glucagon but weakly with extracts of human small bowel mucosa known to

contain enteroglucagon. The degree of cross-reaction was negligible at the sensitive end of the standard curve and became significant only when large and unphysiological amounts of enteroglucagon were added. The assay was performed in 100 µl total volume containing 40% plasma and 100 units aprotinin in duplicate tubes. The porcine pancreatic glucagon standards (Novo) contained an equivalent amount of time-expired blood bank plasma which has been found to be glucagon free. The assay was incubated at 4° C for 5 days. The antibody-bound radioactive glucagon was separated from free radioactive glucagon by addition of 10 mg charcoal as a 1 ml suspension followed by rapid centrifugation and separation of the supernatant. When 4.5 pg of radioactive glucagon label (specific activity 320 µCi/mg) was added, a 10% change in percentage label bound to antibody was produced by plasma containing 80 pg glucagon per ml. Thus the assay could routinely detect changes of 25 pg/ml with 95% confidence.

Results

At delivery the level of plasma glucagon in the normal infants was greater than in their mothers, but this difference was not significant (Table). The umbilical arteriovenous difference was also not significant, and the infants delivered by caesarean section did not differ from those having a vaginal delivery. The glucagon levels were significantly higher in infants with fetal distress than either their mothers ($P < 0.01$) or the normal infants ($P < 0.01$). The mean birthweight was less in the infants with fetal distress, but birthweight alone does not influence cord levels of glucagon in infants of equivalent gestation (Bloom and Johnston, 1972).

TABLE
Infant and maternal plasma glucagon and blood glucose levels

	Mode of delivery	Infant				Mother				
		Umbilical artery		Umbilical vein		Peripheral vein				
		Glucagon (pg/ml)	Glucose (mg/100 ml)	Glucagon (pg/ml)	Glucose (mg/100 ml)	Glucagon (pg/ml)	Glucose (mg/100 ml)			
Normal infants	Vaginal	138 ±14 (30)	62 ±4 (30)	140 ±12 (44)	76 ±3 (44)	122 ±13 (44)	98 ±4 (44)			
	Caesarean	142 ±21 (10)	67 ±5 (10)	130 ±16 (12)	81 ±5 (12)	126 ±17 (12)	105 ±4 (12)			
Hypoxic infants	Vaginal	252 ±33 (5)	60 ±4 (5)	239 ±27 (8)	68 ±4 (8)	131 ±10 (8)	93 ±4 (8)			
	Caesarean	254 ±31 (9)	73 ±4 (9)	248 ±31 (12)	82 ±7 (12)	132 ±13 (12)	106 ±3 (12)			
Normal infants 2 hr after birth	Vaginal			Peripheral vein						
2 hr increment or decrement				188 ±14 (30) +51 ±8 (30)	58 ±2 (30) -23 ±5 (30)	83 ±11 (30) -38 ±6 (30)	87 ±4 (30) -14 ±3 (30)			

Note: Values given are with SEM and the number of subjects in brackets. The 2-hour increment or decrement compares the level at birth in infants' umbilical vein, or maternal peripheral vein, with the level 2 hours after birth.

In the 2 hours after delivery the difference in the glucagon level between the mothers and infants in the normal group became highly significant. The former showed a fall of 38 ± 6 pg/ml ($P < 0.05$) whereas the latter had a rise of 51 ± 8 pg/ml ($P < 0.01$). The rise in glucagon in the infants occurred during a period of falling blood glucose. The mothers also had a fall in blood glucose probably related to discontinuation of intravenous dextrose. There was thus a rise in maternal plasma glucagon during the strenuous phase of labour. This has been more clearly defined by serial measurements in several mothers throughout labour (D. I. Johnston, S. R. Bloom, and K. R. Greene, unpublished observations, 1972).

Discussion

It is apparent from our data that the level of pancreatic glucagon in the human neonate is low and the rise 2 hours after birth relatively small. A highly sensitive assay, using small plasma volumes, was required for these measurements and that is why there are few data in the literature on neonatal glucagon levels. Degradation of plasma glucagon can occur (Mirsky, Perisutti, and Davis, 1959) and may be very rapid, particularly if red cell lysis has caused release of proteolytic enzymes and other substances (Steinke, Gries, and Driscoll, 1967). All samples were therefore protected by addition of the proteolytic enzyme inhibitor aprotinin (Unger *et al.*, 1967) and rapid freezing. The presence of plasma alters antibody binding (Heding, 1971), and therefore the glucagon standards contained glucagon-free plasma in the same proportion as the unknowns. The cross-reaction of enteroglucagon in this assay is minimal. A few patients' samples were assayed in duplicate with Dr. Unger's antiserum 30K which is reported to be highly specific for pancreatic glucagon (Muller, Faloon, and Unger, 1971), and nearly identical glucagon changes were detected.

In our study the absence of significant differences between umbilical artery and vein levels (Table) suggests that the placenta plays little part in fetal glucagon metabolism. We have previously shown that there is no transfer of natural glucagon across the human placenta at term even when the gradient is extremely great (Johnston *et al.*, 1972). The finding of nearly identical levels of glucagon in mother and fetus is therefore probably explained by similar levels of metabolites and also by the fact that both are under some stress.

There are few other reports of the measurement of plasma pancreatic glucagon levels in the human neonate and none include the period of birth and first

few hours after. Fekete *et al.* (1972) catheterized the umbilical vein of normal and premature infants and then exposed them to cold stress. They report a mean level of 390 pg glucagon/ml plasma in the premature infant and 349 pg glucagon/ml plasma in the term infant. Cold stress did not significantly alter these values. This study can be criticized on two points of methodology. Firstly, the infants were studied 4 to 6 hours after a meal when plasma enteroglucagon may have been raised and thus been detected with their antibody which was said to cross-react weakly with this material; and secondly, no proteolytic enzyme inhibitor was added to protect the glucagon from plasma degradation.

In a study of infants with hyperbilirubinaemia (Milner *et al.*, 1972b; Milner, Fekete, and Assan, 1972a) requiring exchange transfusion, high plasma glucagon levels were found. These were not suppressible with glucose and no information was given on the effects of raised bilirubin levels on the assay. No proteolytic enzyme inhibitor was added to the samples. Luyckx *et al.*, (1972) have reported that human portal plasma glucagon values between the 6th and 24th hours of life range from 290 to 4800 pg/ml, tend to rise towards the 24th hour, and are not suppressable by glucose. The only published studies of the glucagon changes in the first 2 hours of life in normal animals are by Girard, Bal, and Assan (1971) in the rat. They found plasma glucagon rises as great as 700 pg/ml, which are of a different order of magnitude from the results in this paper. However, glucagon in the rat may have a more important role in inducing gluconeogenic enzymes (Philippidis and Ballard, 1970), whereas these rate-limiting steps are developed at an earlier stage in human gestation (Räihä and Lindros, 1969).

The ability of young mammals to withstand anoxia is related to their cardiac and hepatic glycogen reserves (Mott, 1961). The term human fetus, when hypoxic, is capable of raising plasma glucose and of accelerating the generation of lactic acid. In their study of normal and hypoxic fetuses at delivery, Stembera and Hodr (1966) measured the plasma glucose gradients between the maternal peripheral vein and the umbilical vein, and also between the umbilical vein and artery. They reported that the former was not significantly affected, but that the latter was reduced and even reversed with increasing degrees of hypoxia. Our study also shows this reduction of the umbilical vein-artery glucose gradient in the stressed fetus. It is not possible to quantitate hepatic glucose release *in vivo*, but

these data suggest that it may be occurring to a significant degree. The much higher glucagon levels found in the anoxic infants suggest that glucagon may be partly responsible for this increased glucose release.

The measurement for the first time of human plasma glucagon at birth has shown the levels to be very similar to those in the human adult. There was a small rise in plasma glucagon occurring together with a fall in blood glucose over the 2 hours after birth. However, there was a much greater glucagon rise associated with the stress of birth anoxia when the blood glucose was normal. This suggests that the blood glucose level may not be the main determinant of neonatal glucagon release. In calves the α -cell is relatively unresponsive to hypoglycaemia in the first 24 hours of life. The calf α -cell has been shown to respond rapidly to splanchnic nerve stimulation (Bloom, Vaughan, and Edwards, 1973). External stress, in the form of pain or fear, has been found to cause large and very rapid glucagon rise in the baboon, and a nervous mechanism of release was postulated (Bloom *et al.*, 1973). It is at least possible that the raised glucagon found in infants with fetal distress is also mediated by the nervous system.

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