Glucagon Deficiency Causing Severe Neonatal Hypoglycemia in a Patient with Normal Insulin Secretion

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Summary

In the presented patient glucagon secretion was not stimulated by hypoglycemia or by infusion of alanine, whereas insulin secretion responded normally to changes in blood glucose and alanine concentrations. The administration of exogenous glucagon evoked an abnormally strong and prolonged hyperglycemic response. Gluconeogenesis was severely impaired, but there was no accumulation of the main gluconeogenic precursors. This was explained by the deficiency of substrates for these precursors, and by shunting into other pathways than gluconeogenesis.

The hypoglycemia was easy to control neonatally, whereas the condition at 3 months of age was critical. Treatment with glucagon resulted in a clinical improvement already observed the first day, whereas blood glucose normalized in 2-3 days. After 1 week the improvement was striking. The prompt initial effect of glucagon was ascribed to increased lipolysis, the delayed glucose rise to an induction of gluconeogenesis. At reexamination the rate of gluconeogenesis had increased 3 times. Glucagon treatment caused significant increase in pyruvate, free fatty acids, and insulin, whereas the alanine concentration fell. The subcutaneous injection of 0.1 mg zinc protamine glucagon per kg body weight resulted in a plasma level of about 900 pg/ml shortly after the injection, with a subsequent exponential fall. The K value was 13.5%/hr.

A brother and a sister probably died from hypoglycemia, and the closely related Pakistani parents had a partly deficient glucagon secretion. An autosomal recessive inherited disorder is suggested.

Speculation

Congenital glucagon deficiency would be expected to cause hypoglycemia, but no such case has so far been well documented. In this paper a patient with severe, persistent, neonatal hypoglycemia caused by an isolated glucagon deficiency is presented. This and similar cases may provide valuable information about the physiologic role of glucagon.

Glucagon, the 29-amino acid hormone secreted by the α_2 cells of the islets of Langerhans in the pancreas, is a most powerful stimulant of hepatic glucose production. Glucagon antibodies injected into fasted rats cause a marked fall in blood glucose concentrations (19), and birds made glucagon deficient die within hours from hypoglycemia (37). In contrast, man has not yet been proved to have an absolute requirement for glucagon. This paper presents a patient with an isolated glucagon deficiency. Treatment with exogenous glucagon was probably essential for his survival.

CASE REPORT AND CLINICAL COURSE

A-B-I, a boy (birth weight 3810 g, length 51 cm), was born on September 25, 1975, 2 weeks later than expected, after an

uneventful pregnancy. His Pakistani parents are close relatives. At the grandparent level brother and sister married sister and brother, and son and daughter from these two couples married each other, becoming parents of the patient to be described. The parents and a brother born in 1973 are living in good health, whereas a sister and a brother, born in 1971 and 1972, respectively, died in Pakistan at 4.5 months and 4 months of age, respectively, with symptoms closely resembling those of the patient. The parents were told that the children had hypoglycemia. The family came to Norway in 1974.

The patient was admitted to the Department of Pediatrics, Rikshospitalet, Oslo, on his third day of life because of repeated attacks of convulsive movements in the upper extremities and cyanosis. He was hypotonic, with a weak cry. Blood glucose was 15 mg/dl, and spinal fluid glucose 8 mg/dl, whereas other routine laboratory findings were normal, including serum electrolytes and liver function tests. Blood ketone bodies were in the lower normal range.

Treatment was successfully started with glucose intravenously, and after a few days normoglycemia was maintained on frequent feedings with extra glucose. At 1 month of age the condition was still good, but single episodes with fasting symptomatic hypoglycemia were recorded. From 1.5-2 months of age scattered attacks of jittering were registered, and mean blood glucose values slowly decreased, despite intensive treatment with varying therapeutic regimens. At 2.5 months he got convulsive attacks, and a previously normal EEG now suggested epileptogenic lesions. The attacks increased in frequency in spite of anticonvulsant treatment. At 3 months of age the condition became critical (Fig. 1). He was hypotonic, semicomatous, nearly continuously hypoglycemic, and an alarming number of seizures were registered. However, all efforts to treat the condition were without success, until glucagon was introduced at 3.5 months of age (Fig. 1).

Except for the addition of glucagon, treatment was unchanged. The first 5 days he received short acting glucagon, 0.1 mg i.m. 6 times daily, thereafter zinc protamine glucagon, 0.4 mg s.c. twice daily. The convulsions ceased abruptly (Fig. 1). After 1 day muscle tonus was definitely increased, preceding the increase in blood glucose, which gradually rose to normal values in 3 days. During the first days of treatment the patient was irritable and weeping, but after 1 week the clinical improvement was striking. There was good tonus, eye contact, and for the first time the patient was smiling.

After 3 weeks the glucagon injections were temporarily discontinued. Two days later blood glucose fell, and the next few days he again got attacks of hypoglycemia. The glucagon therapy was resumed and the patient was rapidly restored (Fig. 1).

In addition to glucagon the patient still needed extra glucose supply, without which blood glucose tended to fall. At 4.5 months of age EEG was normal, and on discharge from hospital at the age of 5.5 months he was in good health with only slightly retarded psychomotoric development. The treatment was continued after discharge, but considering the severe hypoglycemia

Fig. 1. The effect of glucagon treatment on mean blood glucose and the number of seizures registered per day. Horizontal bars: short acting glucagon indicated by vertical cross hatching, zinc protamine glucagon by oblique cross hatching. Vertical bars: numbers of seizures per day.

before glucagon treatment, and because of difficult social conditions at home, we were not too confident with respect to the long term prognosis.

MATERIALS AND METHODS

Short acting glucagon and zinc protamine glucagon were obtained from Novo Industri A/S, Denmark. The first batches of zinc protamine glucagon were generous gifts. L-[U- 14 C]Alanine was purchased from the Radiochemical Centre, Amersham. Enzymes used in the analyses were purchased from Sigma Chemical Company.

Plasma was obtained from heparinized venous blood which was immediately put into an ice bath and separated by centrifugation at 4°. To prevent degradation of glucagon, Trasylol (500 K.I.E./ml) was added to blood intended for glucagon analysis.

Blood glucose was determined by the method of Hultman (26). Plasma alanine and pyruvate were analyzed microfluorometrically by a slight modification of the method of Karl *et al.* (27). Free fatty acids were determined as described by Trout *et al.* (45), and plasma triglycerides according to the "Biochemical Test Combination" (Boehringer, Mannheim).

Amino acids in plasma and urine were determined with a conventional ion exchange procedure at the Department of Clinical Chemistry, Rikshospitalet, Oslo.

All investigations were performed with the parents' consent.

ANALYSES OF HORMONES

Plasma immunoreactive pancreatic glucagon was determined according to the method of Heding (25), and Novo Research Institute kindly supplied us with the pancreas glucagon specific antiserum K 964, glucagon for standards, and [125 I]glucagon. All samples were assayed in triplicate. Recoveries of glucagon added to blood ranged from 89–109%, and the interassay variation of four determinations in a plasma sample containing 59 pg glucagon/ml did not exceed the range of ± 7 pg/ml. To determine the concentrations of nonspecific interfering plasma factors some samples were treated as described by Weir *et al.* (53). The glucagon values presented have not been corrected for possible nonspecific interference.

Immunoreactive insulin was determined by the method of Hales and Randle (22), with kits supplied by the Radiochemical Centre, Amersham. 17-Hydroxycorticosteroids were determined at the Division of Endocrinology, Medical Department B, Rikshospitalet, Oslo, and at the Hormone and Isotope Laboratory, Aker Hospital, Oslo. Catecholamines in urine were determined at the Department of Clinical Chemistry, Rikshospitalet, Oslo.

TOLERANCE TESTS

Secretion of pancreatic glucagon and insulin was evaluated by determining plasma concentrations in the basal state, and in response to infusion of L-alanine, a potent stimulus of glucagon secretion (39, 54). After obtaining a fasting blood specimen, a solution of L-alanine (0.25 g/kg body weight) was injected intravenously during 1–2 min, and blood sampled at intervals. The results were compared with the results of the same test in six patients (from 7 months to 3.5 years old) with hypoglycemia, where disorders of insulin and glucagon secretion had been excluded. Fasting blood glucose values prior to the test were similar in the patient and the control subjects (Fig. 2).

The standard glucagon tolerance test was performed by injecting 0.02 mg glucagon/kg body weight rapidly intravenously, and the intravenous glucose tolerance test by rapid injection of 0.5 g glucose/kg body weight. The tests were performed in the morning after fasting periods varying between 5 and 12 hr, according to the tolerance of fasting.

ASSAY OF GLUCONEOGENESIS

The *in vivo* method employed is based upon the appearance of radioactivity in glucose after intravenous injection of trace amounts of 14 C-labeled gluconeogenic substrates (5 μ Ci/kg body weight). Details of the method and its application are given elsewhere (48–50). Briefly summarized, L-[U- 14 C]alanine was rapidly injected intravenously. At intervals blood was sampled, and plasma was analyzed for the amount of radioactivity in alanine, lactate, and glucose by thin layer chromatography. The results are presented as percentages of the total amount of [14 C]alanine injected, taking different distribution volumes into account. The incorporation of 14 C into glucose was used as an index of the overall gluconeogenesis. The four normoglycemic patients used as control subjects have been described previously (48). The studies were performed with the parents' consent, and the ethical aspects of isotope studies have been discussed in previous publications (48, 49).

STATISTICS

The level of significance was calculated using Student's *t*-test for two means or the paired *t*-test, as judged appropriate, and a *P* value of 0.05 was chosen as the significance level. *K* values for disappearance rates of injected compounds were calculated after computing the least squares fit for an exponential function of the form: $C_1 = C_0 e^{-kt}$, where C_0 is the concentration at time zero, and C_1 the concentration at time *T*. The baseline values were subtracted. The insulin to glucagon molar ratio was calculated by the formula: [insulin (μ U/ml)/glucagon (pg/ml)] $\times 23.3$.

Fig. 2. Changes in blood glucose, insulin, and glucagon after the rapid infusion of alanine at zero time. The patient is compared with six hypoglycemic control subjects (mean $\pm$ SE). I: before glucagon treatment. II: after discontinuation of glucagon treatment for 4 days.

RESULTS

SECRETION OF GLUCAGON AND INSULIN

Despite profoundly depressed blood glucose values, the basal plasma glucagon concentrations were low (Table 1). Moreover, 75% of the low glucagon concentrations apparently found could be shown to be caused by nonspecific plasma interference, not related to pancreatic glucagon. The basal insulin values, on the other hand, were normal.

The glucagon and insulin secretion was further studied after stimulation with alanine. The alanine tolerance test was performed on two occasions: I) at 2.5 months of age, before glucagon treatment was started, and II) at 4 months of age, after discontinuation of glucagon treatment for 4 days. After the infusion of alanine, plasma alanine concentrations increased to 30–40 times baseline values after 5 min, both in the patient and the control subjects, and there was no significant difference in the subsequent decrease. Nevertheless, the glucagon secretion in the patient did not increase, in contrast to all of the control subjects, where the rise was rapid and highly significant ($P < 0.005$) (Fig. 2). The insulin response, however, was brisk, with a maximal increase within the range of the control subjects. Blood glucose decreased in the patient in contrast to the control subjects.

Insulin secretion was also studied in response to glucose. An intravenous glucose tolerance test was performed when the patient was 2 months old. The injected glucose disappeared rapidly, the K value being 7.5 %/min, a high value at this age (24). However, plasma insulin increased only from 7 μ U/ml in the basal state to 14 μ U/ml after the injection of glucose. At 1.5 months of age the response to glucose given perorally was studied (2.5 g/kg body weight). Basal blood glucose was 50 mg/dl, fasting plasma insulin 7 μ U/ml. After glucose was given, blood glucose rose to 78 mg/dl at 30 min, thereafter the glucose values oscillated around the basal value. An insulin maximum of 23 μ U/ml was obtained 15 min after glucose was given.

17-OH-corticosteroids in urine and plasma and the response to ACTH stimulation and to the metyrapone test were normal. The excretion of catecholamines in urine was normal.

RESPONSE TO EXOGENOUS GLUCAGON

The response to exogenous glucagon was studied by a standard glucagon test. The test was repeated four times between 1

and 2 months of age. In Figure 3 the blood glucose response of the patient is compared with the responses of two hypoglycemic control subjects. In contrast to the normal responses of the controls, the blood glucose increase in the patient was abnormally strong and sustained, with a long lasting and high plateau.

STUDIES OF GLUCONEOGENESIS

The patient's gluconeogenesis was measured twice: the first time the patient was 2 months 3 weeks old, *i.e.*, before the glucagon treatment was started; the second time he had been treated with glucagon for 2 weeks, and the study was performed 2 hr after 0.4 mg zinc protamine glucagon had been injected subcutaneously. The basal blood glucose value was 20 mg/dl before the first study, and 100 mg/dl before the second. The basal plasma alanine concentrations were 213 and 141 μ mol/liter, and the pyruvate concentrations 23 and 63 μ mol/liter, respectively. These concentrations were minimally changed by the injection of tracer.

[14 C]Alanine was injected intravenously as described. Before glucagon treatment the incorporation of 14 C into glucose was reduced to one-seventh that of the control subjects, pointing to a severe impairment of gluconeogenesis (Fig. 4). After glucagon

Fig. 3. Blood glucose during standard glucagon tolerance tests. ●: four repeated glucagon tolerance tests (mean $\pm$ SE); ○: one of these tests continued up to 180 min. The patient is compared with two hypoglycemic control subjects with normal responses to exogenous glucagon.

Table 1. Fasting values of some main hormones and metabolites before glucagon treatment, during glucagon treatment, and after discontinuation of glucagon treatment¹

	Glucose, ^{2,3} mg/dl	Insulin, μ U/ml	Glucagon, ⁴ pg/ml	Insulin/ glucagon ratio ⁴	Alanine, μ mol/liter	Pyruvate, μ mol/liter	FFA, ⁵ μ mol/liter	Triglycerides μ mol/liter
I: Before glucagon treatment	34.4 $\pm$ 4.2 (7) I vs. II: $P < 0.005$	9.6 $\pm$ 1.5 (7) I vs. II: $P < 0.05$	55 (2)	5.28 (2)	213 $\pm$ 13.6 (7) I vs. II: $P < 0.025$	31 $\pm$ 8.7 (7) I vs. II: $P = 0.05$	313 $\pm$ 31 (3) I vs. II: $P < 0.05$	730 $\pm$ 122 (6) I vs. II: N.S.
II: During glucagon treatment	69.7 $\pm$ 10.4 (6) II vs. III: N.S.	14.8 $\pm$ 1.5 (6) II vs. III: $P = 0.05$	398 (2)	0.84 (2)	151 $\pm$ 12.7 (3) II vs. III: $P < 0.025$	72 $\pm$ 23.1 (3) II vs. III: N.S.	588 $\pm$ 88 (3) II vs. III: N.S.	518 $\pm$ 48 (3) II vs. III: N.S.
III: Discontinuation of glucagon treatment	53.7 $\pm$ 3.7 (3) I vs. III: $P = 0.025$	9.7 $\pm$ 0.9 (3) I vs. III: N.S.	52 $\pm$ 5.8 (3)	4.35 (3)	318 $\pm$ 45.6 (3) I vs. III: $P < 0.02$	49 $\pm$ 19.6 (3) I vs. III: N.S.	454 (2) I vs. III: $P < 0.05$	636 $\pm$ 48 (3) I vs. III: N.S.

¹ The values are given as mean $\pm$ SE. Numbers of determinations in parentheses.

² I vs. II: The values before (I) and during (II) glucagon treatment (etc.) tested for significance of difference.

³ N.S.: not significant.

⁴ The glucagon values were not corrected for nonspecific interference.

⁵ Free fatty acids.

Fig. 4. Time course for the incorporation of ^{14}C from labeled alanine into plasma lactate and glucose. A tracer dose was rapidly injected intravenously at zero time. The values are presented as percentages of the injected radioactivity. The patient was compared with the mean of four normoglycemic control subjects, the vertical bars showing the range. The patient was studied before glucagon treatment, and after being treated for 2 weeks. Zinc protamine glucagon was injected 2 hr before the last study.

treatment the amount of ^{14}C incorporated into glucose was increased 3 times, but the amount was still below the range of the control subjects.

The disappearance of ^{14}C from plasma alanine was delayed before glucagon treatment, but normalized after treatment. In contrast, the amount of ^{14}C in lactate was close to that of the control subjects both before and after glucagon treatment.

PLASMA AMINO ACIDS

The plasma amino acids at 4 days of age were compared with the data of Brodehl and Gellissen (5). The concentrations of aspartate ($38\ \mu\text{mol/liter}$), serine ($203\ \mu\text{mol/liter}$), glycine ($323\ \mu\text{mol/liter}$), and methionine ($47\ \mu\text{mol/liter}$) were high, whereas there were low levels of alanine ($178\ \mu\text{mol/liter}$), valine ($71\ \mu\text{mol/liter}$), isoleucine ($7\ \mu\text{mol/liter}$), and leucine ($40\ \mu\text{mol/liter}$). The following amino acids were present in normal amounts: threonine, asparagine, glutamate, glutamine, proline, cystine, tyrosine, phenylalanine, ornithine, lysine, histidine, and arginine. In urine glycine was increased ($5.53\ \mu\text{mol/mg creatinine}$). At 3 months of age the amino acids in plasma had normalized, but the excretion of glycine in the urine was still high.

EFFECTS OF GLUCAGON TREATMENT ON FASTING VALUES OF GLUCAGON, INSULIN, AND SOME MAIN METABOLITES

The mean fasting values of glucagon, insulin, and some main metabolites were compared before glucagon treatment, during glucagon treatment, and at the end of the period when glucagon treatment was discontinued (Table 1). The rise in blood glucose during treatment was highly significant ($P = 0.005$). Despite an increase in the insulin values, the insulin to glucagon molar ratio fell. There was a significant fall in plasma alanine, a well known effect of glucagon (8, 9), whereas pyruvate rose. A decrease in plasma triglycerides was indicated, but not statistically significant, whereas free fatty acids increased significantly. In the period when glucagon treatment was temporarily discontinued, the measured values tended to approach the pretreatment concentrations, except for alanine, which increased to values significantly higher than before treatment was started.

KINETICS OF EXOGENOUS LONG ACTING GLUCAGON

The patient, having no endogenous glucagon production, gave the opportunity to study the kinetics of exogenous longacting

Fig. 5. Plasma glucagon and blood glucose after the subcutaneous injection of zinc protamine glucagon ($0.1\ \text{mg/kg}$ body weight) at the time points indicated. The values are presented in a semilogarithmic plot. ●: plasma glucagon; □: blood glucose.

glucagon given subcutaneously. When the patient was 4.5 months old, $0.1\ \text{mg}$ zinc protamine glucagon/kg body weight was injected subcutaneously as shown in Figure 5. In the semilogarithmic plot the glucagon values represented a straight line, and the pairs of data showed a good fit to the equation $C_t = C_0 \cdot e^{-Kt}$ (see under "Materials and Methods"). The correlation coefficient (r) was close to 1.000 and the correlation was highly significant ($P < 0.001$). The calculated disappearance rate, K value, was $13.5\%/hr$, which implies that the glucagon concentration would fall from $935\ \text{pg/ml}$ shortly after the injection to $186\ \text{pg/ml}$ 12 hr after the injection.

The blood glucose values tended to parallel the glucagon concentrations in the semilogarithmic plot. It could further be shown (not displayed) that plasma alanine, and possibly pyruvate, were also correlated with the glucagon concentration, whereas there was no correlation with the measured insulin values.

STUDIES IN PARENTS

Since the case history suggested an inherited disorder, the parents were studied in the fasting state and during an alanine tolerance test.

The mother received $0.22\ \text{g}$ and the father $0.24\ \text{g}$ L-alanine/kg

Table 2. Plasma glucagon, insulin, and glucose concentrations in parents of patient¹

Subjects	Glucagon, pg/ml ²		Insulin, μ U/ml		Glucose, mg/dl	
	Basal	Maximal increment ³	Basal	Maximal increment	Basal	Maximal increment
Healthy controls ⁴	89 $\pm$ 10	108 $\pm$ 12	10 $\pm$ 1	14 $\pm$ 2	78 $\pm$ 2	6 $\pm$ 1
Patient's mother	44	49	15	14	85	-7
Patient's father	56	36	12	11	94	-18

¹ The results are presented as mean $\pm$ SE. The parents were studied in the basal state and after an alanine infusion.

² The glucagon values were not corrected for nonspecific interference.

³ Maximal increment above basal values.

⁴ The results in 21 control subjects published by Donowitz *et al.* (10).

body weight. The results were compared with those published by Donowitz *et al.* (10), who performed a similar alanine tolerance test in 21 healthy nonobese volunteers, but with 0.15 g L-alanine/kg body weight. The analytic methods used differed from those of the present study. With these reservations in mind, it may be inferred from Table 2 that the differences with respect to insulin were minor, whereas the glucagon values in the basal state appeared lower in the parents than in the control subjects. The increment in glucagon secretion after stimulation of the α_2 cells with alanine appeared to be blunted. Furthermore, there was no increase in the blood glucose values, but a late decrease, most apparent in the father.

DISCUSSION

The present report demonstrates clearly that glucagon deficiency will cause severe hypoglycemia in a patient with normal insulin secretion.

The diagnosis of glucagon deficiency was not based mainly on low basal glucagon concentrations, but on the lack of response to hypoglycemia and alanine infusion, both known to be potent stimulators of glucagon secretion (16, 39, 40, 54). This lack of response was demonstrated on repeated occasions, and contrasted the highly significant increase in the control subjects (Fig. 2). Glucagon deficiency thus has to be included in the list of known causes of neonatal persistent hypoglycemia, and should be sought for in all cases of hypoglycemia with obscure etiology. Moreover, regarding the good results of treatment, glucagon secretion should be examined at an early stage.

The disorder was congenital, and the family history and the findings in the parents suggest an inherited disorder with an autosomal recessive mode of inheritance. We have no pancreas biopsy of the patient and do not know the morphologic correlate to the disorder, but the normal insulin secretion points to a defect specifically involving the pancreatic α_2 cells, leaving the β cells unaffected. The existence of extrapancreatic α_2 cells is disputed in man (3, 38) in contrast to other species such as the dog (34, 51).

The biologic role of glucagon is to ensure, in concert with insulin, a steady supply of substrates under a wide range of physiologic conditions. The molar ratio of insulin to glucagon seems to be more important than the absolute concentration of either (46). In the patient described the most significant effect of the unopposed insulin action was the severe hypoglycemia, the cause of which might be deficiencies in three main metabolic processes providing energy-yielding substrates, namely gluconeogenesis, glycogenolysis, and lipolysis (31).

Gluconeogenesis normally starts after birth (13, 23) by the normal postnatal increase of glucagon (17, 43) inducing the hepatic enzyme phosphoenolpyruvate carboxykinase, one of the key enzymes of gluconeogenesis (7, 23, 42). In later life glucagon is an important stimulator of gluconeogenesis in situations of glucose need (8, 12, 13). The severely impaired gluconeogenesis in the presented patient was considerably improved by glucagon treatment, but not quite normalized (Fig. 4). However, high blood glucose concentrations have been shown to depress gluconeogenesis in both animals and in man (15, 35), and blood

glucose in our patient rose 5-fold from the first to the second study, during the second study exceeding the mean blood glucose values of the controls by more than 40% (48).

Glucagon is a potent hepatoglycogenolytic agent (31), whereas insulin stimulates the synthesis of glycogen. In our patient the abnormally strong and prolonged hyperglycemia provoked by the standard glucagon tolerance test suggested great deposits of glycogen in the liver, caused by the unopposed insulin action (Fig. 3). The response further indicated excessive sensitivity to exogenous glucagon, as would be expected in a patient with no glucagon production of his own.

Glucagon stimulates lipolysis and ketogenesis (29, 32), thereby liberating free fatty acids, glycerol, and ketone bodies. Ketone bodies may largely substitute for glucose as fuel for the brain (11, 41), and free fatty acids are a main energy source for the liver, thus being a prerequisite for the energy-requiring process of gluconeogenesis (6). Furthermore, glycerol is rapidly converted to glucose in the liver (1). In our patient the concentrations of free fatty acids and ketone bodies were in the lower normal range before glucagon treatment, and could not compensate for the low blood glucose concentrations. The glucagon treatment caused a significant rise in free fatty acids (Table 1), thus providing the patient with additional energy-yielding substrates.

Main precursors for gluconeogenesis are lactate and the glucogenic amino acids, the majority of these precursors flowing through pyruvate (13, 14). In our patient the concentrations of lactate, pyruvate, and alanine were low despite the severely impaired gluconeogenesis. Although the other glucogenic amino acids tended to be high, alanine is the major precursor in this context (14). However, in contrast with the other glucogenic amino acids, alanine is chiefly synthesized *de novo* from pyruvate in extrahepatic tissues by the transamination of pyruvate (14). Low concentrations of pyruvate limit substrate availability for this transamination. Reduced protein catabolism caused by the glucagon deficiency (9, 14) decreases the amount of amino groups to be transaminated. The low levels of pyruvate and lactate were probably caused by the deficiency of glucose, their main precursor (28). Additionally, glucagon inhibits pyruvate oxidation (8, 13, 21). Consequently, glucagon deficiency would be expected to increase oxidation of pyruvate and thereby reduce the levels of gluconeogenic precursors.

The hypoglycemia in the presented patient was initially easy to control, whereas the condition at 3 months of age was virtually resistant to conventional treatment. Varma *et al.* (47) demonstrated that dogs under 55 days of age were less sensitive to insulin action than were adult dogs. The disappearance rate of glucose in man is low the first neonatal days, increases to a maximum between 4 and 12 months of age, and then slowly decreases towards adult levels (24). A decreased glucose utilization during the first weeks of life might explain the clinical course of our patient.

Glucagon treatment resulted in a definite clinical improvement the first day of treatment, 2 days before blood glucose had normalized. This observation may be explained by the lipolytic effect of glucagon, rapidly increasing the concentrations of free fatty acids and ketone bodies, whereas normalization of blood

glucose depends on the induction of gluconeogenesis. This induction has been shown to have a lag period of 2–4 days in man (13, 33, 43).

Our patient was treated with zinc protamine glucagon, administered subcutaneously in the amount of 0.1 m/kg body weight twice daily. Plasma glucagon is normally low and shows small fluctuations during the day (44). The plasma concentrations in our patient showed great variations. The mean glucagon concentrations, however, might not be far from physiologic, as concentrations in the portal circulation are higher than in peripheral blood (2), and glucagon is mainly acting on the liver. Too high plasma glucagon concentrations should be avoided, as they may cause harmful catabolic effects (30).

Hypoglycemia in children has been attributed to glucagon deficiency on several occasions (18, 20, 52) since the original description by McQuarrie *et al.* (36) of presumed α cell deficiency in familial infantile hypoglycemia. These reports were based upon histologic evidence of a reduced number of pancreatic α cells, but did not take into account that only a small proportion of the α cells is comprised of α_2 cells, being alone capable of secreting glucagon, and the deficiencies were not verified by measurements of pancreatic glucagon. So far a deficiency of immunoreactive pancreatic glucagon in hypoglycemia has been reported only once in a young adult male (4). However, in this case, there was deficient secretion of both insulin and glucagon. This complicated the clinical picture. Furthermore, similar deficiencies are seen in unspecific islet cell destructions (10).

CONCLUSION

A Pakistani boy with a family history of infant deaths was admitted shortly after birth because of hypoglycemic seizures. After some initial improvement, his condition became critical with recurrence of the seizures. Despite severe hypoglycemia, basal glucagon values were low, and did not increase after alanine infusion. Insulin secretion was normal. Gluconeogenesis was severely impaired. The hyperglycemic response to a standard glucagon tolerance test was abnormally strong. Treatment with glucagon resulted in immediate cessation of the seizures, whereas blood glucose increased to normal values in 3 days. Gluconeogenesis increased 3-fold. After 1 week of glucagon treatment the clinical condition had strikingly improved, and after 5 weeks a previously abnormal EEG had normalized. Discontinuation of glucagon treatment resulted in a relapse. The closely related parents also had low glucagon values, responding weakly to stimulation with alanine. An autosomal recessive inherited disorder is suggested. This is the first case described with normoinsulinemic glucagon deficiency as the cause of persistent neonatal hypoglycemia.

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Arginase	hyperammonemia
argininosuccinate synthetase	hyperlysinemia
homocitrullinuria	

Recently we (17) reported an 18-year-old mentally and physically retarded boy, suffering from anorexia and vomiting from 2 months of age. Our case closely resembles Colombo's, but the specific findings of the former were abnormally high concentra-