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Hepatic Ultrastructure in Leprechaunism

Hepatic Ultrastructural Evidence Suggesting a Syndrome with Defective Hepatic Glucose Release

Sanford I. Roth[1], H.K. Schedewie[2], Dennis M. Bier[4], H. Herschel Conaway[3], Jerrold Olefsky[5], Arthur Rubenstein[6], and M. Joycelyn Elders[2]

Departments of Pathology[1], Pediatrics[2] and Physiology[3], The College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR 72205,
[4] Departments of Medicine, Washington University, School of Medicine, St. Louis, MO
[5] University of Colorado School of Medicine, Denver, CO
[6] University of Chicago School of Medicine, Chicago IL, USA

Summary. Leprechaunism is a congenital syndrome with characteristic habitus and facies, with fasting hypoglycemia and hyperinsulinism. In response to a glucose challenge there is prolonged severe hyperglycemia with an increased hyperinsulinemia. Our studies on such a patient showed a normal response of the serum glucose to glucagon stimulation in the fed state but no response in the postabsorptive state. Ultrastructural studies on the hepatocytes demonstrated that a lack of hepatic glycogen was not responsible for the biochemical features, since there was abundant normal β-glycogen in both the fed and fasting state, the granules being smaller in the fasted state. We speculate that carbohydrate intolerance in leprechaunism may be due to a relative insulin resistance of cell receptors in the fed state. Reactive hyperinsulinemia persisting into the postabsorptive phase appears to antagonize the usual glycogenolytic response to glucagon during fasting, resulting in hypoglycemia despite the presence of large hepatic glycogen stores.

Key words: Leprechaunism – Glycogen – Liver – Glucagon – Hypoglycemia

Characteristic features of leprechaunism, a rare inherited syndrome of infants (Donahue 1948), include intrauterine growth retardation, typical facies, phallic enlargement, marked deficiency of subcutaneous fat stores and striking cutaneous abnormalities. All of the patients studied have had postprandial hyperglycemia and hyperinsulinism, insulin resistance and severe fasting hypoglycemia (Kobayashi et al. 1978). Leprechaunism thus differs

Offprint requests to: S.I. Roth, Department of Pathology, Northwestern University, Medical School 303 E. Chicago Ave, Chicago, IL 60093 USA

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from other hyperinsulinemic syndromes such as congenital generalized lipodystrophy (CGL) (Seip 1971) and acanthosis nigricans with insulin resistance (ANIR) (Kahn et al. 1976) which are associated with normal or elevated levels of fasting blood glucose (Seip 1971 and Kahn et al. 1976), CGL has many of the features of leprechaunism including genital hypertrophy, acanthosis nigricans, and hypertrichosis (Fernandes et al. 1969), but the disease differs from leprechaunism by being associated with increased growth and muscle mass and hepatosplenomegaly (Seip 1971). ANIR, type B, has antiinsulin receptor antibodies (Kahn et al. 1976), not found in patients with leprechaunism (D'Ercole et al. 1979) while Type A has defective insulin receptors and presents with accelerated growth and virilization (Field 1979).

Earlier glucose turnover studies in patients with leprechaunism suggested that the disordered carbohydrate metabolism was caused by a combination of insulin resistance and an accelerated fasting state with rapid depletion of hepatic glycogen (Bier et al. 1980). Ultrastructure studies presented in this report on the liver of one of these patiens in both the fasted and fed state call for modification of this hypothesis regarding the pathogenesis of the biochemical alterations observed in leprechaunism.

Materials and Methods

This was a 3 year old white female with leprechaunism whose clinical and other features have been previously reported in detail (Kobayashi et al. 1978). She weighed 2,600 g at term, and she was phenotypically a leprechaun. She had progressive acanthosis nigricians (Kobayashi et al. 1978; Roth et al. 1981). Repeated evaluations revealed the typical biochemical features of leprechaunism, such as fasting hypoglycemia (<40 mg/100 ml) with hyperinsulinemia (>100 μU/ml), as well as prolonged, severe hyperglycemia with an increased hyperinsulinemia (>2,000 μU/dl) in response to glucose loading (Bier et al. 1980). The plasma glucagon levels were elevated (>7,100 pg/ml) during fasting but declined appropriately with glucose loading in both the fed and fasting state. Her insulin responses to intravenous tolbutamide, glucose, galactose and glycerol were markedly exaggerated (Kobayashi et al. 1978; Bier et al. 1980).

Intravenous glucagon stimulation tests following a short (4 h) and a prolonged (12 h) fast were performed using 0.05 mg/kg of glucagon injected over one minute. Blood samples were collected for the measurement of glucose and insulin over a 60 min period of time. A needle biopsy of the liver was obtained after a 13 h fast, when the serum glucose was 38 mg/dl, lactate 1.3 meq/liter and the insulin 790 μU/ml. There was insufficient material for glycogen assays. An open liver biopsy and a muscle biopsy were obtained after 4 continuous hours of infusion of 10% glucose in water at a rate of 7 mg/kg/min. At this time the serum glucose was 384 mg/dl and the insulin 2,060 μU/ml. The hepatic and muscle glycogen content on these specimens were normal (5.7% and 0.96% of wet tissue weight, respectively (Bier et al. 1980).

Both liver biopsies were fixed in 2.5% gluteraldehyde in Cacodylate (Karnovsky 1965) buffer (pH 7.4) and postfixed in Caulfield's (1957) modification of Palade's (1952) veranol buffered osmium tetroxide (pH 7.4). In addition a portion of the open biopsy was fixed in 2.5% gluteraldehyde in 0.1 M Sorenson's (Lillie 1965) phosphate buffer (pH 7.4) prior to postfixation in Caulfield's (1957) and primary fixed in Caulfield's (1957) osmium tetroxide. All were fixed at room temperature. The tissues were dehydrated through graded alcohols and embedded in epon 812 after the method of Luft (1961). Seventy to 80 nm sections were cut on the LKB ultramicrotome III, with a diamond knife, mounted on copper grids and examined in a Sieman's Elmiskop 102 at 80 Kv after staining with uranyl acetate (Watson 1958) and lead citrate (Reynolds 1963; Venable and Coggeshall 1965). Glycogen granule diameters were measured on plates at magnifications of 37,450 and 17,700 diameters calibrated with a grid containing 21,600 lines/cm.

Results

Glucagon Stimulation. Following a 4 h fast the plasma glucose was 40 mg/dl and the serum insulin was 855 μU/ml (Normal 6–26 μU/ml). Glucagon injection caused an elevation of the plasma glucose to 90 mg/dl within 15 min while the serum insulin showed a typical biphasic response reaching a maximum of 5,858 μU/ml after 5 min (Table 1). After a 12 h fast the plasma

Table 1. Response to glucagon (0.75 mg intravenously) after fasting short term – 4 h or long term – 12 h

Time (min)	4 h fasting		12 h fasting	
	Glucose (mg/dl)	Insulin (μU/ml)	Glucose (mg/dl)	Insulin (μU/ml)
0	40	855	24	114
2	54	1,930	24	72
5	64	5,858	24	59
10	82	4,885	24	117
15	98	5,441	24	155
30	98	2,790	25	81
45	82	1,779	21	40
60	78	1,169	22	40

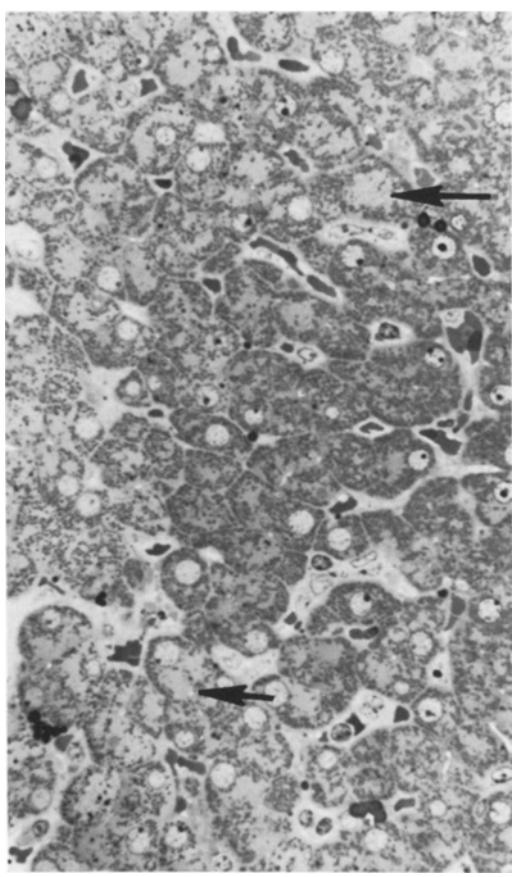

Fig. 1. Thin section of epon-embedded section of fasting liver specimen. The clear cytoplasmic areas (*arrows*) represent glycogen. Toluidine blue. × 490

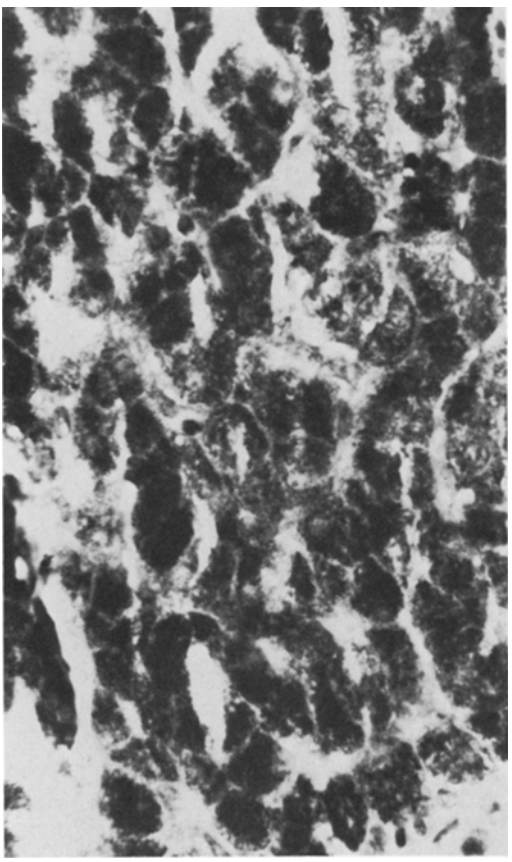

Fig. 2. Glycogen filled liver cells after feeding. Periodic acid Schiff. ×490

glucose fell to 25 mg/dl and glucagon failed to elicit any glycemic response. Serum insulin levels remained elevated (114 μU/ml) and had a moderate biphasic response to the glucogen injection (Table 1).

Light Microscopy. Histological studies of the needle biopsy on 1 μm sections of the fasting liver showed no diagnostic abnormalities (Fig. 1). There was abundant PAS-positive material in the hepatocyte cytoplasm. The postprandial open biopsy was without histological abnormality. Abundant PAS-positive, diatase-digestible material, presumbly glycogen filled the hepatic cells (Fig. 2). There was no cirrhosis or inflammation and no recognizable abnormality of the portal triads, central veins, hepatocytes or Kupfer cells.

Electron Microscopy. There were no significant differences between the various methods of fixation. In the fed state the hepatic cells were unremarkable (Figs. 3 and 4). The nuclei were centrally located with an occasional nucleolus. β-glycogen granules were present in the nuclei (Fig. 3). The cell membranes were straight and unremarkable. Terminal bars surrounded the canaliculi. The cytoplasm contained normal hepatic mitochondria with calcium

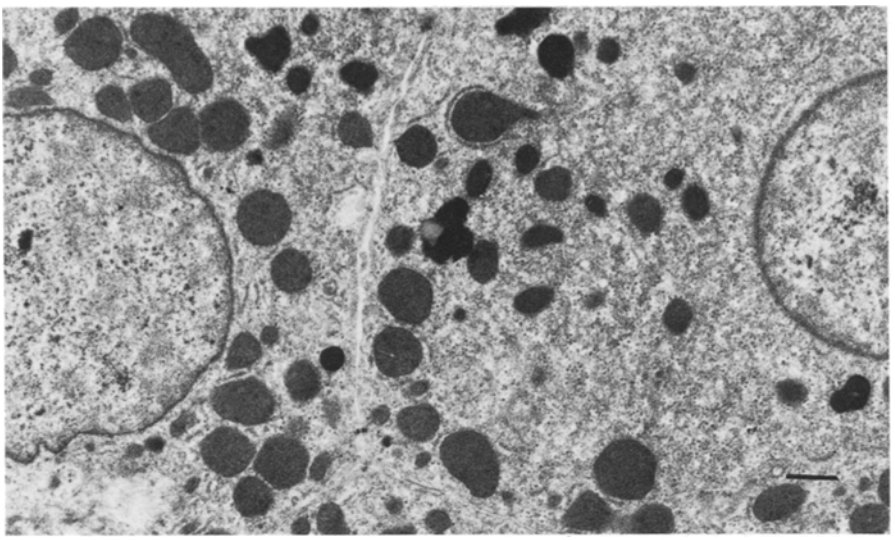

Fig. 3. Hepatocytes from the fed liver filled with abundant glycogen between other normal organelles. The nuclei contain glycogen granules. Lead citrate and uranyl acetate. ×1,565

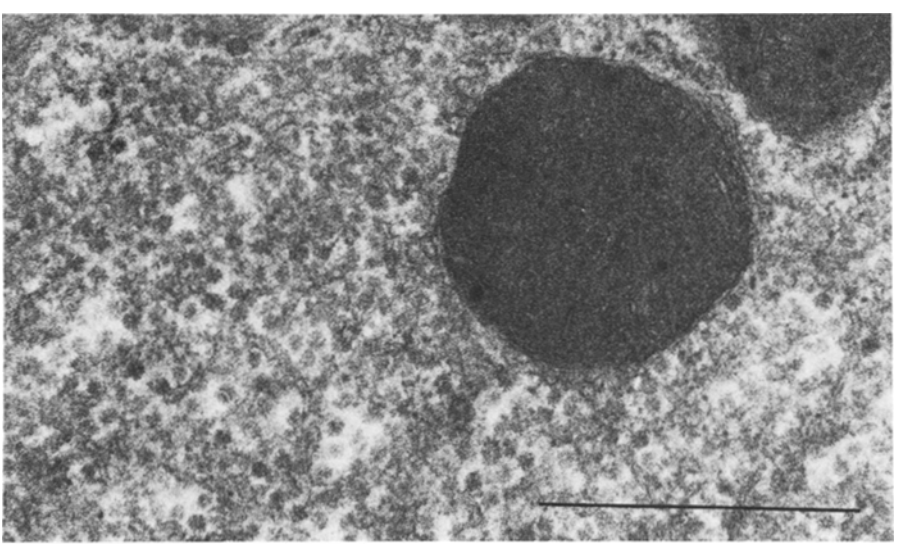

Fig. 4. β-subunits of glycogen between mitochondria filling the cytoplasm of a fed hepatocyte. Lead citrate and uranyl acetate. ×43,435

bodies. Smooth and rough endoplasmic reticulum, Golgi regions, microtubules, lipid bodies, peroxisomes and lysosomes were all within normal limits. The remainder of the cytoplasm was filled with normal appearing individual β-granules of glucogen measuring 62.2 ± 9.6 (mean $\pm$ standard deviation) nm in diameter (Drochmans 1962). A few rosettes of α-granules were present.

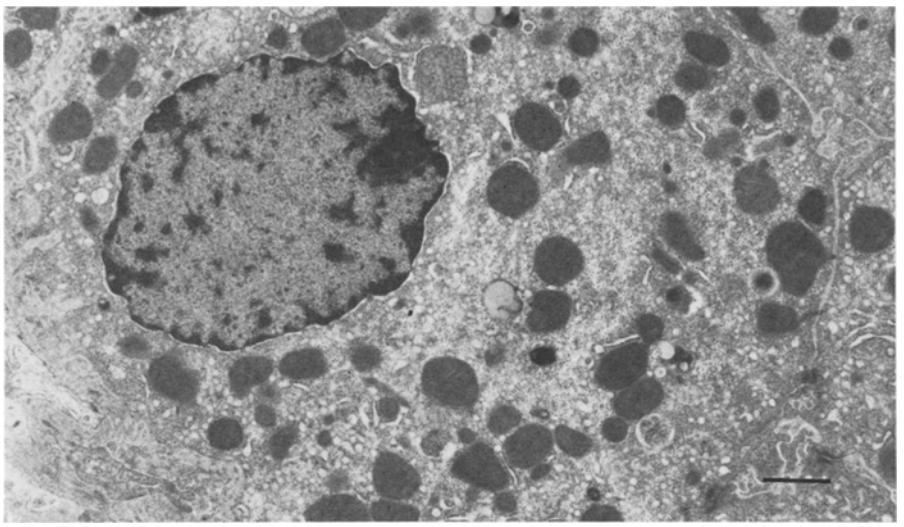

Fig. 5. Hepatocytes from the fasted liver are filled with glycogen. Other organelles are within normal limits. Lead citrate and uranyl acetate. ×1,565

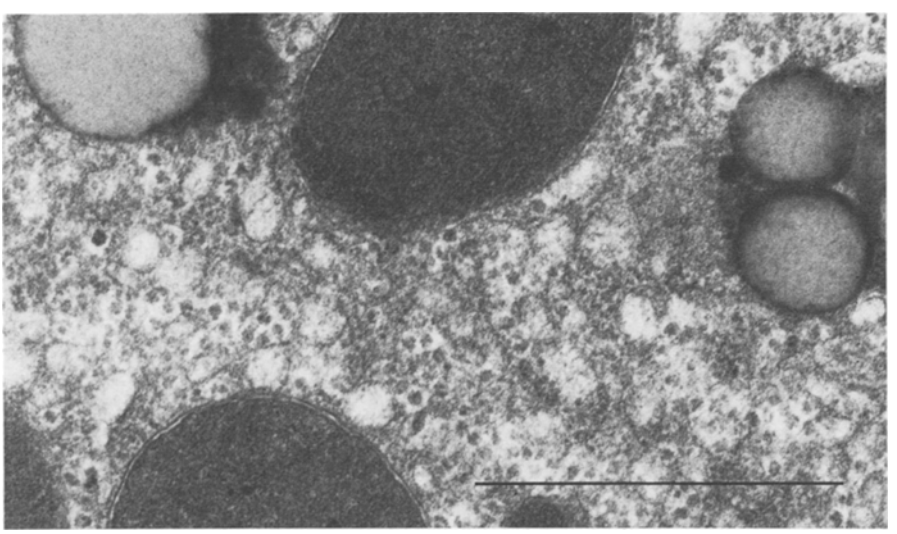

Fig. 6. β-subunits of glycogen and lipid droplets between mitochondria filling the cytoplasm of the hepatocyte after a fast. Lead citrate and uranyl acetate. ×48,190. Marker on all electron micrographs equals 1 μm

The ultrastructure of the fasted liver was almost indistinguishable (Figs. 5 and 6) from that of the fed liver. In the areas intervening between the usual organelles, these cells were also filled with individual β-granules of glycogen measuring 51.2 ± 6.6 nm in diameter with a few rosettes of α-granules. Due to the single type of fixation it was not possible to evaluate the presence of nuclear glycogen. The mean diameter of the β-glycogen

granules in the fasted liver was significantly different from that of the fed liver at the 0.01 level. In contrast to Pompe's disease (Baudhuin et al. 1964; Bruni and Paluello 1970) no glycogen was seen in membrance-limited vesicles, in either the fed or fasted liver. No abnormal inclusions of fibrous glycogen as seen in amylopectinosis (Schochet et al. 1970) were noted in either biopsy.

Discussion

Leprechaunism is a syndrome parts of which are characteristic biochemical findings including fasting hyperglycemia and hyperinsulinemia (Bier et al. 1980). It can be distinguished from most of the other fasting hypoglycemias such as primary hyperinsulinism, hepatic dysfunction, panhypopituitarism, ketotic hypoglycemia, impaired gluconeogenesis or neoplasm (Arky 1979).

In our patient the glycemic response to an intravenous glucagon challenge was normal in the fed state but absent after 12 h of fasting. For comparison, normal children show an average glycemic response of 32 ± 6.2 mg/dl after a fast as long as 26–28 h (Finegold et al. 1980). In children with insulin-induced hypoglycemia the glucose increment after glucagon is 71 ± 10.5 mg/dl and in children with ketotic hypoglycemia 8.1 ± 2.4 mg/dl (Finegold et al. 1980). A normal response to glucagon in the fed state and the failure of glucagon to increase blood glucose levels after fasting has been observed in patients with panhypopituitarism, severe liver disease, defects in glycogen synthesis or release, type III glycogen storage disease or a prolonged fast (Fernandes et al. 1969; Brown and Brown 1978; Garancis et al. 1970; Hopwood et al. 1975; Stanley and Baker 1976; and Howell 1978). At autopsy only a variety of non-specific hepatic alterations have been described, including excess glycogen, excess iron storage, and giant cells (Donahue 1948; Salmon and Webb 1963; Ordway and Stout 1973). However, the lack of information on the feeding state of these infants at the time of death and the time post mortem make these studies irrelevant to the arguments presented here.

A defect in glycogen synthesis or an inborn error of glycogenolysis (the glycogenoses) could explain the fasting hypoglycemia and the difference in the response to a glucagon challenge in the fed and fasting state (Kobayashi et al. 1978; Seip 1971; Garancis et al. 1970; Howell 1978). The β-glycogen granules in our patient like those in patients with type III glycogenosis, Cori's disease (Finegold et al. 1980), are larger than those of the normal liver (Drochmans 1962), and further are significantly larger in the fed than in the fasted liver. However, no cases of hepatomegaly or excess glycogen storage have been reported in leprechaunism. The fed glycogen content of our patient's liver and muscle were biochemically within normal limits (Bier et al. 1980) and hyerinsulinemia is not seen in Cori's disease. Further, an autopsy performed in our laboratory on a similar patient, a 6 month old black female weighing 2,230 g (1st percentile), revealed a 75 g liver (normal for the patient's body weight, but small for the patient's age), without significant glycogen content at the light microscopic level. Neither

was there excess glycogen in the muscles or other organs, nor has excess glycogen storage been reported in these organs in other leprechauns. An assay of the enzymes controlling glycogenolysis in the liver and muscle were within normal limits (Bier et al. 1980).

An acceleration of the deplation of hepatic glycogen that normally occurs slowly after a prolonged fast has been proposed to explain the fasting hypoglycemia (Bier et al. 1980), and would explain the glucagon response. However, since we found a normal hepatic glycogen content at the time that the patient had the most severe hypoglycemia, this is not a tenable hypothesis.

A close, inverse relationship between ambient insulin concentration and receptor concentration has been demonstrated in several states of altered insulin sensitivity (Kahn et al. 1976; Olefsky 1976; Bar and Roth 1977; and Soll et al. 1975). Furthermore, studies of insulin receptors on cultured cells have shown that this alteration in insulin receptor concentration is a direct effect of insulin (Gavin et al. 1974; Kosmakos and Roth 1976; and Schilling et al. 1979). Monocyte and fibroblast receptor binding studies on our patient after an 18 h fast were found to be normal (Kobayashi et al. 1978). However, erythrocyte insulin receptor binding after 4–6 h fasting have been consistently low (Herzberg et al. 1980). Unfortunately we were not able to obtain sufficient material for the study of hepatocyte insulin receptors.

It would thus appear that the moderate hyperinsulinemia and increased insulin receptor concentration during prolonged fasting may be sufficient to overcome the insulin resistance and inhibit hepatic glucose output after glucagon prior to glycogen depletion even in the presence of hypoglycemia. The ability of glucagon to stimulate hepatic glucose production in the fed state regardless of 10–100 fold elevated insulin concentration, may be due to a "down regulation" of insulin receptors in the fed state and inhibition of liver glycogenolysis by a relatively moderate excess of insulin could account for the inappropriate preservation of hepatic glycogen stores during fasting in patients with leprechaunism.

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