Proc. Natl. Acad. Sci. USA
Vol. 76, No. 11, pp. 5877–5881, November 1979
Medical Sciences

Primary defect of insulin receptors in skin fibroblasts cultured from an infant with leprechaunism and insulin resistance

(intrauterine growth retardation/genetic defect/polypeptide hormone)

ELLEN E. SCHILLING*, MATTHEW M. RECHLER*, CARL GRUNFELD†, AND ALAN M. ROSENBERG‡

*Laboratory of Biochemical Pharmacology and †Diabetes Branch, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20205; and ‡Department of Pediatrics, University of Manitoba, Winnipeg, Manitoba, Canada

Communicated by Elizabeth F. Neufeld, July 5, 1979

ABSTRACT **Insulin binding to insulin receptors on skin fibroblasts established in culture from an infant with insulin resistance and clinical features of leprechaunism was markedly decreased in comparison with cultures from an age-matched control. By contrast, the binding of epidermal growth factor, a polypeptide growth factor chemically unrelated to insulin, to patient's and control fibroblasts was indistinguishable. The selective defect in insulin binding to patient's fibroblasts was reflected in an impaired ability of insulin to stimulate 2-deoxyglucose uptake. These results most likely indicate a primary genetic defect of insulin receptors.**

The binding of a hormone to its receptor is thought to be the first step in hormone action (1). Strong support for this concept has come from the direct demonstration that certain genetic syndromes of hormone resistance in humans are associated with primary abnormalities of receptors (reviewed in ref. 2). Examples include the androgen receptor in testicular feminization (3) and other forms of androgen resistance (4, 5) and the low density lipoprotein receptor in familial hypercholesteremia (6).

This report describes a primary defect of insulin receptors occurring in an infant with leprechaunism (7) and insulin resistance. Secondary derangements of insulin receptors have been reported on circulating cells and adipocytes in several states of insulin resistance and hyperinsulinemia including obesity (8), glucocorticoid excess (9), acromegaly (10), adult-onset diabetes mellitus (11), in some patients with lipoatrophic diabetes (12, 13), and in two groups of patients with circulating immunoglobulins directed against their insulin receptors—namely, patients with extreme insulin resistance type B (14, 15) and with ataxia telangiectasia (16). Skin fibroblasts propagated in culture are not subject to *in vivo* humoral influences such as hyperinsulinemia or antireceptor antibodies and more faithfully reflect the genetic endowment of the donor (3–6). Fibroblast cultures established from skin from normal volunteers possess insulin receptors with the characteristic specificity for insulins of different biological potencies seen in other insulin receptors (17). In the present study, we demonstrate a profound, selective deficit of insulin receptors and impaired stimulation of glucose uptake by insulin in skin fibroblasts established in culture from an insulin-resistant patient with leprechaunism.§

MATERIALS AND METHODS

Case Description. A complete clinical description of the patient will be reported elsewhere.¶ In brief, the patient was a Canadian Indian male infant who weighed 2.41 kg (3rd percentile) at birth. His parents were first cousins once removed. His physical appearance was considered to be typical of leprechaunism: emaciation, absence of subcutaneous fat, decreased muscle mass, hirsutism, and low-set, poorly developed ears. The clinical course was marked by failure to thrive, pneumonia of unknown etiology, and sudden death at 47 days of age. Additional clinical findings included tachycardia, cardiomegaly, a small patent ductus arteriosus, transient neonatal tyrosinemia responsive to vitamin C, mild hyperbilirubinemia, proteinuria, and aminoaciduria.

The most striking clinical feature was profound hyperinsulinemia and insulin resistance. At age 17 days, blood glucose was 40 mg/100 ml after a 6-hr fast, with a coincident insulin level (by radioimmunoassay) of 660 μunits/ml (normal fasting insulin, 15 μunits/ml). Of 11 fasting blood glucose determinations, 4 showed hypoglycemia, 3 showed borderline hypoglycemia, 3 gave normal results, and 1 showed hyperglycemia. Insulin was strikingly increased in four of four analyses (660–2480 μunits/ml), with concomitant normal (twice), borderline (once), and hypoglycemic (once) blood glucose values. After feeding, mild hyperglycemia (181–248 mg/100 ml) and hyperinsulinemia (600, 2600 μunits/ml) were observed. Fasting-state serum was fractionated on Sephadex G-50 and showed 87% insulin and 13% proinsulin (a normal distribution) without anti-insulin antibodies. The biological activity of the circulating insulin determined by glucose oxidation in rat adipocytes was 70% of normal (relative to immunoreactivity). At postmortem examination, marked hypertrophy of the pancreatic β cells was noted.

The patient's mother was hyperinsulinemic despite normal blood glucose levels. His two siblings are normal. The mother's family history included two babies with unusual facies who died in early infancy.

Fibroblast Cultures. Fibroblast cultures were established in Winnipeg from the patient (skin from upper arm, day 20) and from a normal male control (foreskin, day 7). After seven and six passages, respectively, cells were shipped to The National Institutes of Health for the studies to be described. They were used after an additional two to seven passages. Both cultures were handled identically for each experiment.

An additional control cell line was used where specified: GM 41 established from the skin of a 3-month-old normal female (obtained from the Human Genetic Mutant Cell Repository, Camden, NJ).

Cell Cultivation. Fibroblast cultures (16, 17) were grown at 37°C in modified Eagle's minimal essential medium supplemented with 10 mM Hepes buffer, nonessential amino acids, neomycin sulfate, and 20% fetal calf serum (Flow, lot 4055838). Cultures were divided 1:3 or 1:4 each week.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviation: EGF, epidermal growth factor.

§ A preliminary report of this work has been presented: Schilling, E. E., Rosenberg, A. M., Grunfeld, C. & Rechler, M. M. (1979) *Program, The Endocrine Society, 61st Annual Meeting,* Anaheim, CA, June 13–15, Abstr. 397.

¶ See *Note Added in Proof.*

Patient's and control cells and media were cultured for mycoplasma (broth culture for 21 days; agar subculture for 14 days) by Microbiological Associates (Bethesda, MD) and were negative.

Insulin Binding to Fibroblasts in Suspension. Pork insulin (crystalline zinc, 25 units/mg) was obtained from Lilly. ^{125}I-Labeled insulin (^{125}I-insulin) was prepared by the stoichiometric chloramine-T method (18). The specific radioactivity of the preparations used was 125–183 Ci/g (1 Ci = 3.7×10^{10} becquerels).

Binding studies were performed as previously described (16, 17). Fibroblasts were inoculated into 100-mm plastic petri plates and used approximately 1 week after feeding. For experiments, fibroblasts were detached from the cell monolayer as described (17) with minor modifications. Cultures were washed with phosphate-buffered saline and incubated for 5 min at 37°C with trypsin (Worthington; 3150 BAEE units/mg; 0.1 mg/ml) and 0.5 mM EDTA. Soybean trypsin inhibitor (Sigma; 0.2 mg/ml) was added, and the cells were sedimented, washed, and resuspended in pH 8.0 Hepes binding buffer (0.1 M Hepes/0.12 M NaCl/1.2 mM $MgSO_4$/2.5 mM KCl/10 mM glucose/10 mg of bovine serum albumin per ml).

The incubation mixture consisted of ^{125}I-labeled insulin (approximately 100 pg/ml), the indicated concentration of unlabeled insulin, and 1–2.5 $\times 10^6$ fibroblasts (as indicated) in a volume of 0.5 ml. Incubation was for 5 hr at 15°C; then, duplicate 0.2-ml aliquots were pipetted into microfuge tubes (0.4 ml) containing 0.2 ml of iced Hepes binding buffer, the tubes were centrifuged in a microfuge B (Beckman) for 1 min, and the supernate was aspirated and discarded. Tips containing the cell pellets were excised and assayed in a gamma spectrometer (Searle) at 82% efficiency.

Nonspecific (or nonsaturable) binding was determined in the presence of a large excess of unlabeled insulin (typically, 10 μg/ml).

Insulin Binding *In Situ*. Fibroblasts were inoculated at high density (2–5 $\times 10^5$ cells per well) in Linbro Multiwell (FB-6-TC) tissue culture plates containing six 35-mm wells; they were used 2–11 days later. Prior to assay, the cell monolayer was washed twice with Dulbecco's phosphate-buffered saline containing Ca^{2+} and Mg^{2+}. The incubation mixture was the same as for the assay using suspended cells except that the final volume was 0.8–1.0 ml. After a 5-hr incubation at 15°C, the incubation medium was aspirated and the cell monolayer was washed three times with cold Hepes binding buffer and solubilized with 1 ml of 0.1% sodium dodecyl sulfate, and the lysates were transferred to counting tubes.

DNA content was determined fluorometrically on the same cell lysates, with calf thymus DNA (Worthington) as a standard (19).

Uptake of 2-Deoxyglucose. One week after plating, fibroblasts were detached from the 100-mm culture plates in the same manner as for insulin binding experiments. Cells were washed twice in 20 vol of Krebs–Ringer phosphate buffer containing bovine serum albumin (20 mg/ml) and $CaCl_2$ (1.3 mM). Cells (5 $\times 10^5$) were incubated in the same buffer (0.225 ml) in the presence or absence of insulin for 20 min. Measurement of transport was started by the addition of 0.025 ml of 2 mM 2-[^{14}C]deoxyglucose (2 μCi/μmol). After incubation for 5 min at 37°C, the reaction was terminated by the addition of 10 vol of Krebs–Ringer phosphate buffer with bovine serum albumin (1 mg/ml) and $CaCl_2$ (1.3 mM). Cells were collected on Whatman GF/C filters and washed four times. Filters were assayed in a liquid scintillation counter at 75% efficiency.

Binding of Epidermal Growth Factor (EGF). The binding of EGF to human fibroblasts was measured as described (20, 21). Unlabeled EGF purified from mouse submaxillary glands and ^{125}I-labeled EGF (^{125}I-EGF) were kindly provided by J. De Larco (National Cancer Institute).

Fibroblasts were grown in 35-mm multiwell plates. The cell monolayers were incubated with ^{125}I-EGF (2 ng/ml; 10 Ci/g) and the indicated concentrations of unlabeled EGF in 1 ml of binding buffer (modified Eagle's medium containing 1 mg of bovine serum albumin per ml and 50 mM BES [*N*,*N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid at pH 6.8] at 37°C for 1 hr. Cells were washed and solubilized as described (21), and cell-associated radioactivity was determined in a gamma spectrometer. Results are expressed per μg of DNA. Cell-associated radioactivity includes both ^{125}I-EGF bound to surface receptors and internalized ^{125}I-EGF–receptor complexes.

Cell Volume. Volumes were determined electronically by M. Cassidy Habberset and K. Hamilton (National Cancer Institute) using a Coulter Counter, model B, interfaced with a Coulter Channelyzer and a logarithmic amplifier (22). The reference particle was a 10-μm-diameter polystyrene microsphere (Particle Technology, Los Alamos, NM). Cell surface area was calculated by assuming the cell to be a sphere.

RESULTS

Insulin Binding. The binding of ^{125}I-insulin to fibroblasts from patient and control was studied after suspension of the cells from the monolayer by gentle trypsinization. Control fibroblasts (from foreskin of a 7-day-old infant) bound 2.9% of the input radioactivity per 10^6 cells, of which 2.3% represented specific or saturable binding (determined in the presence of excess unlabeled insulin) (Fig. 1). Half-maximal inhibition of specific binding occurred at approximately 2 ng of unlabeled insulin per ml. These results are similar to those obtained with fibroblast cultures of normal adult skin (16, 17, 23) and with cultures of skin from a 3-month-old normal female infant (GM 41) (results not shown).

By contrast, the binding of ^{125}I-insulin to the patient's fibroblasts differed in several respects. Total binding was lower

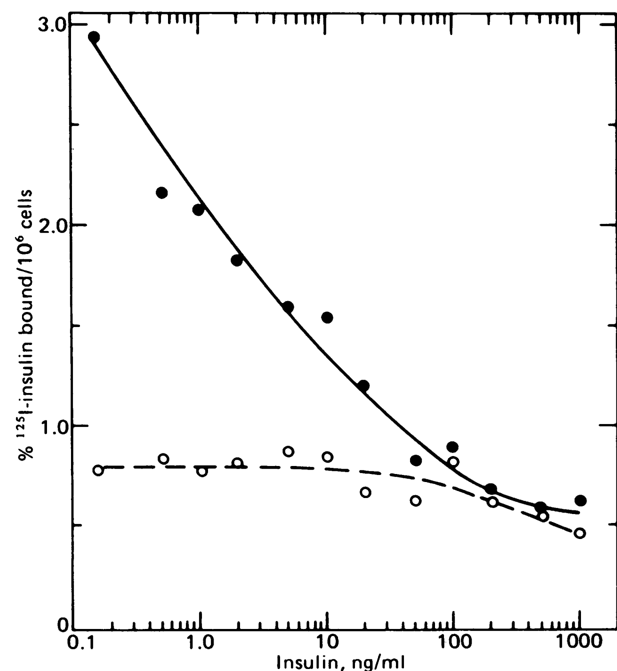

FIG. 1. Insulin binding to fibroblasts in suspension. Fibroblasts from patient (○---○) and control (●—●) were suspended by trypsinization. Incubation was for 5 hr at 15°C with 4.0 $\times 10^6$ control cells or 5.0 $\times 10^6$ patient's cells per ml.

(<0.8%/10^6 cells), and inhibition by excess unlabeled insulin was minimal (≈0.35%/10^6 cells) and was observed only at concentrations of unlabeled insulin >100 ng/ml.

The impaired insulin binding to patient's fibroblasts did not result from degradation of the ^{125}I-insulin tracer. Incubation of ^{125}I-insulin with patient's fibroblasts, control fibroblasts, or buffer alone under binding assay conditions in the absence of unlabeled insulin did not decrease the fraction of tracer precipitated by 10% trichloroacetic acid or capable of binding to the insulin receptors of the IM-9 human lymphoblastoid cell line (1) (data not shown). In addition, when the binding of ^{125}I-insulin to patient's fibroblasts was examined at times from 0 to 5 hr (not shown), maximal binding was observed at 5 hr (the incubation time used in the experiment in Fig. 1) and no earlier transient peak of binding was detected.

Decreased binding of insulin to patient's fibroblasts also was found when ^{125}I-insulin was bound directly to fibroblast monolayers, without exposure of the cells to trypsin (Fig. 2). The dose-response curve obtained with control fibroblasts *in situ* was similar to that obtained with suspension cultures of control cells: half-maximal inhibition of binding of ^{125}I-insulin occurred at 2–3 ng of insulin per ml. Once again, low total binding and negligible specific binding of ^{125}I-insulin to patient's fibroblasts was observed. Thus, the low binding to patient's cells observed in suspension assay did not result from increased susceptibility of the patient's insulin receptors to trypsin.

The decreased insulin binding to patient's fibroblasts cannot be attributed to a major difference in cell size. The surface area of patient's fibroblasts, estimated by use of the Coulter Channelyzer, was only slightly smaller than that of control fibroblasts (848 μm^2 compared with 1017).

Decreased binding of insulin to patient's fibroblasts was found when patient's and control cells were grown to the same density (Fig. 1) and when patient's cells were grown to a lower density (not shown). Quiescent cultures were used for all binding experiments.

In order to determine whether the decreased insulin binding to patient's fibroblasts was a selective deficit or part of a more generalized membrane alteration, the binding of another polypeptide growth factor, EGF, chemically different from insulin, was examined. EGF is a potent mitogen for human fibroblasts in culture, and human fibroblasts possess abundant receptors for EGF (20, 21). ^{125}I-EGF was incubated with monolayer cultures of fibroblasts from patient and control for 1 hr at 37°C (Fig. 3). Cell-associated radioactivity [which includes both ^{125}I-EGF bound to cell surface receptors and internalized EGF–receptor complexes (20)] was determined. Analysis of the competitive binding data as a dose–response curve for inhibition (Fig. 3*A*) or as a saturation plot (Fig. 3*B*) did not reveal significant differences between patient's and control cells.

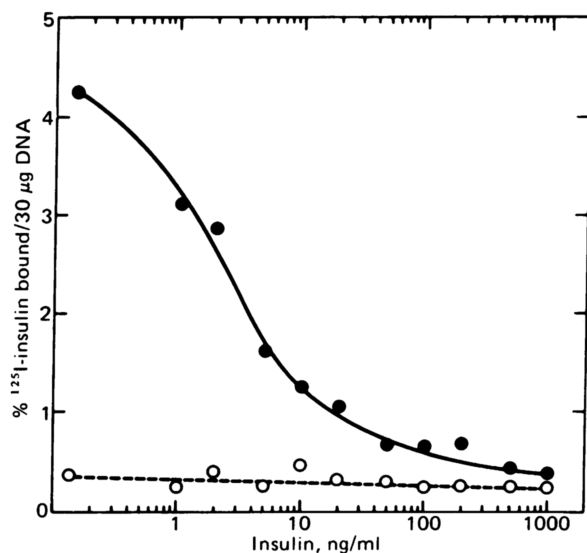

FIG. 2. Insulin binding to fibroblasts *in situ*. Patient's (O- - -O) or control (●—●) cells were seeded 11 days prior to assay in tissue culture plates containing 35-mm wells. Incubation was for 5 hr at 15°C. Each point is the mean of duplicates. The mean DNA content of patient's cells was 28 μg and has been adjusted to the mean DNA content of the control cells, 30 μg, for comparison.

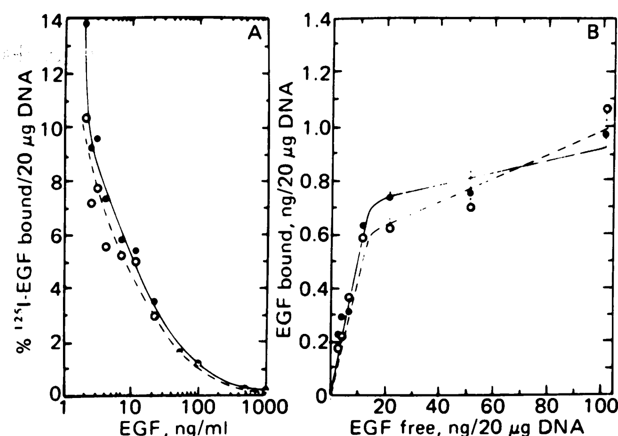

FIG. 3. EGF binding to fibroblasts *in situ*. Fibroblasts were used 18 hr after plating 4 × 10^5 patient's cells per 35-mm well (O- - -O) or 6 × 10^5 control cells per 35-mm well (●—●). The mean DNA content per well was 16.8 μg for patient's fibroblasts and 28 μg for control fibroblasts. (*A*) EGF includes ^{125}I-EGF and unlabeled EGF. Mean of duplicate points is shown. (*B*) Data from *A* replotted as bound EGF versus free EGF. The range of duplicates in the plateau region is shown.

2-Deoxyglucose Uptake. We next asked whether the selective defect in insulin binding to insulin receptors on patient's fibroblasts was functionally significant. Stimulation of 2-deoxyglucose uptake by insulin at 1 μg/ml was examined in fibroblast cultures after suspension by trypsin treatment. In the experiment shown in Fig. 4, uptake by control cells was stimulated ≈50% by insulin, whereas insulin did not enhance the uptake by patient's fibroblasts. In five experiments, the stimulation in control fibroblasts was 56% ± 7% (SEM), whereas the stimulation in patient's fibroblasts was 5% ± 8% (SEM).

DISCUSSION

In this infant with physical features of leprechaunism, insulin resistance was indicated by persistent excessive hyperinsulinemia (600–2600 μunits/ml) with intermittent, mild hypoglycemia. The patient's endogenous circulating insulin was normal in both size and biological activity in an *in vitro* glucose oxidation bioassay. His serum did not contain anti-insulin antibodies. The patient was too ill to tolerate challenge with exogenous insulin.

The insulin receptor defect in our patient was expressed in fibroblast cultures established from a skin biopsy specimen and maintained for many generations in culture. The profound impairment of insulin binding was observed when fibroblasts were studied *in situ* or after suspension by trypsin treatment, in the absence of insulin degradation, at different culture densities and after different times of incubation. Because the residual insulin binding was very low and the inhibition by excess unlabeled insulin was minimal, it was not possible to determine whether the defect was exclusively in the concentration of insulin receptors, or whether their affinity also was affected. By contrast, binding of EGF, a polypeptide growth

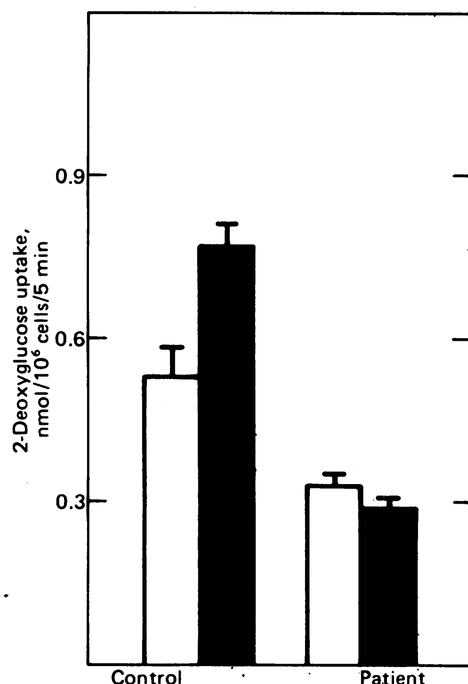

FIG. 4. Insulin stimulation of 2-deoxyglucose uptake. Fibroblasts were removed from the culture plates by trypsin treatment and suspended in the presence (■) or absence (□) of insulin (1 μg/ml) for 20 min at 37°C. Cells were then incubated in 0.2 mM 2-[^{14}C]deoxyglucose (2 μCi/μmol) for 5 min and collected on filters. Uptake by control and patient's fibroblasts is shown. A control for trapping was performed by mixing cells with labeled deoxyglucose and immediately filtering; this control value has been subtracted for each data point. The mean ± SEM of triplicate determinations is shown.

factor chemically unrelated to insulin, to fibroblasts cultured from our patient was normal. Thus, decreased insulin binding probably reflects a selective deficit of insulin receptors rather than a generalized membrane abnormality. Stimulation of 2-deoxyglucose uptake by insulin, an acute metabolic activity thought to be mediated by the insulin receptor, also was impaired in our patient's fibroblasts.

We have demonstrated a primary and selective defect of insulin receptors in our patient. Abnormal insulin binding secondary to metabolic or immunologic alterations has been described in both acquired and genetic disorders with insulin resistance: obesity (8), some cases of lipoatrophic diabetes (12, 13), extreme insulin resistance with acanthosis nigricans Type B (14, 15), and ataxia telangiectasia (16). However, in those instances in which fibroblast cultures were established so that insulin binding could be studied free from humoral influences—lipoatrophic diabetes (24), ataxia telangiectasia (16), and insulin resistance Type B (23)—the insulin binding defect was not observed. Thus, although some component of the insulin resistance in our patient might have been secondary to hyperinsulinemia or antireceptor antibodies, the fact that the receptor defect was expressed on fibroblasts in culture that were not exposed to these humoral influences excludes these possibilities.

The clinical features of leprechaunism are sufficiently nonspecific that a heterogeneous group of patients are undoubtedly included within this diagnosis. In the 24 reported cases (including ours) and 2 cases reported in abstract, abnormal glucose homeostasis has been frequently described. Hypoglycemia and beta-cell hyperplasia have been most common. Hypoglycemia is difficult to evaluate in emaciated infants without stores of fat or glycogen. At least one patient with leprechaunism was unable to mobilize glucose upon infusion of epinephrine (25). Beta-cell hyperplasia may indicate hyperinsulinemia, but increased insulin levels have been documented only in seven cases (refs. 26–31 and the present case). Resistance to exogenous insulin was described in three cases (28, 29), and insulin resistance was inferred in two cases (ref. 31 and the present case). Insulin binding has been studied in four of these patients. D'Ercole *et al.* (31) reported normal insulin binding to fibroblasts. Kobayashi *et al.* (29) reported normal insulin binding to both circulating monocytes and cultured fibroblasts; however, insulin-stimulated glucose transport appeared to be abnormal in their patient's fibroblasts. Hill (30) described decreased insulin binding to erythrocytes from one of the two siblings studied by Jacobs *et al.* (28); binding to fibroblasts has not been reported. Thus, within the group of presumed leprechaunism patients, there is a clear-cut (albeit heterogeneous) subset with documented insulin resistance. Within this subgroup, insulin receptors appear to be normal in two patients [a postreceptor defect has been documented in one patient (29) and inferred in one (31)], and abnormal in two patients (ref. 30 and our patient). Whether the insulin receptor defect in the patient cited by Hill (30) will prove to be primary (as in our patient) or secondary awaits the results of fibroblast binding experiments.

In general, one would anticipate that defects of insulin receptors and insulin resistance would be manifested clinically as glucose intolerance. However, if the insulin receptor defect were expressed *in utero*, the initial manifestation might well be intrauterine growth retardation. Insulin is thought to be a major hormonal determinant of fetal growth. Patients with islet cell agenesis (30, 32) have extremely low birth weight and decreased subcutaneous fat and muscle, and they die shortly after birth. Similarly, these features in our patient might well be the expression of an insulin receptor defect *in utero*. Insulin may play a crucial role in the synthesis of fat, glycogen, and muscle protein *in utero*. Moreover, given an emaciated infant with depleted fat and (presumably) glycogen stores, it is not surprising that fasting hyperglycemia was observed infrequently and that fasting hypoglycemia was noted, as well as mild postprandial hyperglycemia. The hyperinsulinemia in our patient also might be an expression of the underlying insulin receptor defect (e.g., a short feedback loop with the insulin concentration sensed by the beta-cell insulin receptor), an expression of insulin resistance at the level of the beta cell, or a function of integrated glucose concentrations throughout the day.

The minimal symptoms of hypoglycemia or glucose intolerance in our patient despite a severe deficit of insulin receptors lead us to suggest that primary genetic abnormalities of insulin receptors might be more widespread than previously appreciated. Severe deficits might be manifested as low birth weight, as in our patient. Screening such infants for hyperinsulinemia and potential insulin resistance would give a more reasonable estimate of the frequency of this abnormality. Milder deficits of insulin receptors might not be appreciated until later life, perhaps as incidental clinical findings. For example, patients with extreme insulin resistance and acanthosis nigricans type A (14, 15)¶ or certain patients with lipoatrophic diabetes (12, 13) might be part of a continuum of primary insulin receptor defects of lesser degrees of severity.

Note Added in Proof. The clinical description has been accepted for publication (33). C. R. Kahn and J. M. Podskalny (personal communication) have recently found an alteration in the binding of insulin to its receptor in fibroblasts cultured from a type A patient with the syndrome of insulin resistance and acanthosis nigricans (14, 15).

We thank Dr. R. Chochinov, whose consultation initiated this collaboration. E.E.S. was supported in part by The Washington D.C. Area Affiliate of the American Diabetes Association.

1. Kahn, C. R. (1976) *J. Cell Biol.* **70**, 261–286.
2. Verhoeven, G. F. M. & Wilson, J. D. (1979) *Metabolism* **28**, 253–289.
3. Keenan, B. S., Meyer, W. J., III, Hadjian, A. J., Jones, H. W. & Migeon, C. J. (1974) *J. Clin. Endocrinol. Metabl.* **38**, 1143–1146.
4. Griffin, J. E., Punyashthiti, K. & Wilson, J. D. (1976) *J. Clin. Invest.* **57**, 1342–1351.
5. Amrhein, J. A., Klingensmith, G. J., Walsh, P. C., McKusick, V. A. & Migeon, C. J. (1977) *N. Engl. J. Med.* **297**, 350–356.
6. Brown, M. S. & Goldstein, J. L. (1976) *N. Engl. J. Med.* **194**, 1386–1390.
7. Donohue, W. L. & Uchida, I. (1954) *J. Pediatr.* **45**, 505–519.
8. Bar, R. S., Gorden, P., Roth, J., Kahn, C. R. & De Meyts, P. (1976) *J. Clin. Invest.* **58**, 1123–1135.
9. Kahn, C. R., Goldfine, I. D., Neville, D. M., Jr. & De Meyts, P. (1978) *Endocrinology* **103**, 1054–1066.
10. Muggeo, M., Bar, R. S., Roth, J., Kahn, C. R. & Gorden, P. (1979) *J. Clin. Endocrinol. Metabl.* **48**, 17–25.
11. Olefsky, J. M. & Reaven, G. M. (1975) *J. Clin. Invest.* **54**, 1323–1328.
12. Oseid, S., Beck-Nielsen, H., Pedersen, O. & Sovik, O. (1977) *N. Engl. J. Med.* **296**, 245–248.
13. Wachslicht-Rodbard, H., Muggeo, M., Saviolakis, G. A., Harrison, L. A., Flier, J. S. & Kahn, C. R. (1979) *Clin. Res.* **27**, 379A (abstr.).
14. Flier, J. S., Kahn, C. R., Roth, J. & Bar, R. S. (1975) *Science* **190**, 63–65.
15. Kahn, C. R., Flier, J. S., Bar, R. S., Archer, J. A., Gorden, P., Martin, M. M. & Roth, J. (1976) *N. Engl. J. Med.* **294**, 739–745.
16. Bar, R. S., Levis, W. R., Rechler, M. M., Harrison, L. C., Siebert, C., Podskalny, J., Roth, J. & Muggeo, M. (1978) *N. Engl. J. Med.* **298**, 1164–1171.
17. Rechler, M. M. & Podskalny, J. M. (1976) *Diabetes* **25**, 250–255.
18. Roth, J. (1975) *Methods Enzymol.* **37**, 223–233.
19. Karlsson, F. A., Grunfeld, C., Kahn, C. R. & Roth, J. (1979) *Endocrinology*, **104**, 1383–1392.
20. Carpenter, G. & Cohen, S. (1976) *J. Cell Biol.* **71**, 159–171.
21. Todaro, G. J., De Larco, J. E. & Cohen, S. (1976) *Nature (London)* **264**, 26–31.
22. Cassidy, M., Fowlkes, B. J. & Herman, C. J. (1975) *Acta. Cytol.* **19**, 117–125.
23. Muggeo, M., Kahn, C. R., Bar, R. S., Rechler, M., Flier, J. S. & Roth, J. (1979) *J. Clin. Endocrinol. Metabl.* **49**, 110–119.
24. Rosenbloom, A. L., Goldstein, S. & Yip, C. C. (1977) *J. Clin. Endocrinol. Metabl.* **44**, 803–806.
25. Dekaban, A. (1965) *Arch. Dis. Child.* **40**, 632–636.
26. Summitt, R. L. & Favara, B. E. (1969) *J. Pediatr.* **74**, 601–610.
27. Hartdgen, R. G., Dogliotti, M., Rabinovitz, L. & Bartlett, R. G. (1975) *Br. J. Dermatol.* **93**, 587–591.
28. Jacobs, R. F., Nestrud, R. M., Beard, A. G., Fiser, R. H., Fawcett, D. D., Hill, D. E., Morris, M. D. & Elders, M. J. (1978) *Clin. Res.* **26**, 71A (abstr.).
29. Kobayashi, M., Olefsky, J. M., Elders, J., Mako, M. E., Given, B. D., Schedwie, H. K., Fiser, R. H., Hintz, R. L., Horner, J. A. & Rubenstein, A. H. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3469–3473.
30. Hill, D. E. (1978) *Semin. Perinatol.* **2**, 319–328.
31. D'Ercole, A. J., Underwood, L. E., Groelke, J. & Plet, A. (1979) *J. Clin. Endocrinol. Metabl.* **48**, 495–502.
32. Dodge, J. A. & Laurence, K. M. (1977) *Arch. Dis. Child.* **52**, 411–413.
33. Rosenberg, A. M., Haworth, J. C., De Groot, G. W., Trevenen, C. L. & Rechler, M. M. *Am. J. Dis. Child.*, in press.