

## Primary cortisol resistance in man. A glucocorticoid receptor-mediated disease.

G P Chrousos, ... , D L Loriaux, M B Lipsett

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**Research Article**

We have studied a man suspected of having primary cortisol resistance on the basis of high 24-h mean plasma cortisol levels (27.4 micrograms/dl) and no stigmata of Cushing's syndrome. His son had slightly elevated 24-h mean plasma cortisol levels (9.9 micrograms/dl; normal 7.52 micrograms/dl). Both had high plasma protein unbound cortisol and increased urinary free cortisol. Plasma ACTH concentration was high, and both were resistant to adrenal suppression by dexamethasone. The father appeared to have mineralocorticoid excess resulting in hypertension, hypokalemia, and metabolic alkalosis. This was found to be due to markedly elevated plasma levels of deoxycorticosterone and corticosterone. The son, who was normotensive, had mildly increased plasma corticosterone and normal deoxycorticosterone levels. To study the apparent end-organ resistance to cortisol, we examined the glucocorticoid receptor in the white cells and fibroblasts of these patients. In both tissues, using both whole cell and cytosol assays, the glucocorticoid receptor was found to have reduced affinity for dexamethasone. In the cytosol assays, a reduced receptor number was found as well. We conclude that cortisol resistance is a rare familial syndrome owing to an abnormal glucocorticoid receptor with a decreased affinity for cortisol.

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# Primary Cortisol Resistance in Man

## A GLUCOCORTICOID RECEPTOR-MEDIATED DISEASE

GEORGE P. CHROUSOS, A. VINGERHOEDS, DAVID BRANDON, CHARLES EIL, MICHEL PUGEAT, MONIQUE DEVROEDE, D. LYNN LORIAUX, and MORTIMER B. LIPSETT, *Developmental Endocrinology Branch, National Institute of Child Health and Human Development, and the Clinical Center, National Institutes of Health, Bethesda, Maryland 20205; National Naval Medical Center and Uniformed Services University of the Health Sciences, Bethesda, Maryland 20205; Ziekenhuis Bethel, Delft, Holland.*

**ABSTRACT** We have studied a man suspected of having primary cortisol resistance on the basis of high 24-h mean plasma cortisol levels (27.4  $\mu\text{g}/\text{dl}$ ) and no stigmata of Cushing's syndrome. His son had slightly elevated 24-h mean plasma cortisol levels (9.9  $\mu\text{g}/\text{dl}$ ; normal 7.52  $\mu\text{g}/\text{dl}$ ). Both had high plasma protein unbound cortisol and increased urinary free cortisol. Plasma ACTH concentration was high, and both were resistant to adrenal suppression by dexamethasone. The father appeared to have mineralocorticoid excess resulting in hypertension, hypokalemia, and metabolic alkalosis. This was found to be due to markedly elevated plasma levels of deoxycorticosterone and corticosterone. The son, who was normotensive, had mildly increased plasma corticosterone and normal deoxycorticosterone levels. To study the apparent end-organ resistance to cortisol, we examined the glucocorticoid receptor in the white cells and fibroblasts of these patients. In both tissues, using both whole cell and cytosol assays, the glucocorticoid receptor was found to have reduced affinity for dexamethasone. In the cytosol assays, a reduced receptor number was found as well. We conclude that cortisol resistance is a rare familial syndrome owing to an abnormal glucocorticoid receptor with a decreased affinity for cortisol.

### INTRODUCTION

Two patients, a father and son, with long term "hypercortisolism" not associated with clinical manifestations of Cushing's syndrome were described by one of us in 1976 (1). The father, with the more severe

hypercortisolemia, also had hypertension and hypokalemic alkalosis. The son had normal blood pressure and serum electrolytes. In this paper we present our studies of the pathophysiologic mechanisms that underlie the resistance to cortisol in these patients and the hypertension and hypokalemic alkalosis found in the father.

### METHODS

**Patients.** The father [patient I, age 58 yr, National Institutes of Health (NIH) 1448481] (height 161 cm, weight 62.2 kg) was noted to have hypercortisolism at the age of 48 yr during evaluation for hypertension (180–190/120–130 mm Hg) and hypokalemic alkalosis. Subsequent studies revealed markedly elevated plasma cortisol levels, increased 24-h urinary 17-ketogenic steroids, and an increased cortisol production rate. There were no stigmata of Cushing's syndrome. The plasma renin activity was normal and 24-h urinary aldosterone values on a 9-g NaCl diet were low. When evaluated 10 yr later at NIH, the patient again showed none of the features of Cushing's syndrome. He had repeatedly normal blood pressure but low serum potassium and elevated bicarbonate. He had been without any anti-hypertensive treatment for 4 wk. Supplemental potassium was given, followed by a gradual rise of serum potassium from 2.2 to 3.5 meq/liter. The 24-h urinary potassium ranged between 86 and 128 meq during the period of hypokalemia. He was always normonatremic (141–146 meq/liter). Renal function tests were normal. Liver function tests were also normal with the exception of serum total protein and albumin that were low (5.4 and 3.3 g/dl, respectively, with normal ranges of 6.1 to 7.7 and 4.5 to 5.3). The hemoglobin was 15.2, hematocrit 42.4%, and lymphocyte count 6,200 (85% polymorphonuclear, 8% lymphocytes, 4% monocytes, and 2% eosinophils). Serum cholesterol and triglycerides were normal. Oral glucose tolerance test indicated carbohydrate intolerance (fasting blood glucose, 51 mg/dl, and serum insulin, 4.9  $\mu\text{U}/\text{ml}$ , 2-h glucose 266, and insulin 33). However this test was performed during hypokalemia severe enough to inhibit insulin release (2).

The son (patient II, age 26 yr, NIH 1448468, height 173 cm, weight 84 kg) was found to have hypercortisolism at the

Address reprint requests to Dr. Chrousos, National Institutes of Health.

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age of 20 during family studies stimulated by the unusual findings in his father. He was asymptomatic. The 17-ketogenic steroids and cortisol production rate were both elevated. During his hospitalization at NIH he was normotensive and had normal serum electrolytes. Urinary potassium excretion ranged between 59 and 83 meq/24 h on a regular hospital diet. The following laboratory tests were within normal limits: complete blood count, serum cholesterol and triglycerides, renal and liver function tests, and glucose tolerance test.

Delayed hypersensitivity tests were performed on both patients. Positive reactions to dermatophyton and mumps were present in both. The father had a positive response to intermediate strength purified protein derivative and a chest x-ray indicative of old calcified granulomatous lesions. Tubercle bacilli could not be seen in or grown from the sputum. The son had a negative response to intermediate strength purified protein derivative.

Adrenal sonography was normal in both patients. There was no radiological evidence of osteoporosis; both men worked as heavy construction workers and had suffered no fractures.

**Hormone assays.** Serum concentration of cortisol (3), corticosterone (4), dehydroepiandrosterone sulfate (5),  $\Delta^4$ -androstenedione (5), 17-hydroxyprogesterone (6), 11-deoxycortisol (3), and insulin (7) were measured by previously described radioimmunoassays. Urinary free cortisol (8) and aldosterone (9) were measured as previously described. We are indebted to Dr. Maria I. New for the serum deoxycorticosterone assays (10), Dr. Dorothy T. Krieger for the ACTH measurements (11), and Dr. Robert Vigersky for the dexamethasone determinations (12).

**Measurement of serum-free cortisol and cortisol-binding globulin capacity and affinity.** The protein-unbound (free) cortisol was measured in duplicate by dialysis at 37°C after 18 h of gentle shaking (13, 14). 1 ml of fivefold diluted serum was dialyzed against 10 ml of phosphate-buffered saline (0.01 M  $\text{PO}_4$ , 0.15 M NaCl, pH 7.4). The concentration of unbound cortisol was calculated using the formula of Slaunwhite (15) corrected for the initial dilution.

The cortisol-binding globulin capacity and affinity were also measured by equilibrium dialysis, using the buffer and conditions mentioned above, on 1:5 diluted and charcoal pretreated serum to remove endogenous steroid (13, 14). The nonspecific binding was assessed according to Chamness and McGuire (16), and the data were analyzed by the Scatchard method (17) using the final cortisol concentration inside the dialysis bag as recommended (18). Patient and normal control samples were studied concurrently.

**Glucocorticoid receptor assays.** We measured glucocorticoid receptor affinity and concentration in two tissues: circulating mononuclear leukocytes and skin fibroblasts cultured in vitro, using both whole cell and cytosol.

The glucocorticoid receptor in mononuclear leukocytes was estimated as described (19). About  $1 \times 10^7$  cells were incubated with [ $^3\text{H}$ ]dexamethasone at six concentrations between 1 and 40 nM for 8 h at 24°C in the presence and absence of 100-fold unlabeled dexamethasone. Specific binding was calculated as the difference between total and nonspecific binding. The data were analyzed using the method of Scatchard. The binding capacity was expressed as fmol/ $10^6$  cells.

Fibroblast strains were established from skin specimens obtained by punch biopsy and were processed as described (20). They were grown to confluence, dispersed, and  $0.4\text{--}1.0 \times 10^6$  cells were placed in end assay tubes. Cells were incubated for 60 min at room temperature with five concen-

trations of tritiated dexamethasone (1–20 nM). Nonspecific binding was determined by the addition of 1  $\mu\text{M}$  unlabeled dexamethasone. The binding capacity was expressed as the number of binding sites per cell.

The binding of [ $^3\text{H}$ ]dexamethasone (six concentrations, 1–20 nM) to the cytosol glucocorticoid receptor was measured using a described assay system (21, 22) utilizing dextrose-coated charcoal to separate bound from free steroids. Cytosol from mononuclear leukocytes obtained by leukopheresis and from cultured skin fibroblasts was prepared as described (22). The former was pretreated with dextran-coated charcoal to remove endogenous steroids, whereas the fibroblasts were grown in serum (cortisol)-free media 48 h before the study. Cytosol protein content was determined by the method of Lowry et al. (23). The buffer that was used for the preparation of fibroblast cytosol contained 50 mM sodium molybdate, giving a final concentration of sodium molybdate of 25 mM during the incubation period. Cytosols from both tissues were otherwise processed similarly.

**Statistical analysis.** Unless otherwise indicated, the results are presented as mean  $\pm$  SE of repeated separate measurements in each patient ( $m$  = number of measurements) and as mean  $\pm$  SD of control values ( $n$  = number of controls). A two-tailed Student's  $t$  test was used for comparison between each patient and the control group, assuming that the variance was only due to the population variability, as measured in the control group (24). The patient group had an  $n_1 = 1$  and the control group an  $n_2 = n$  as indicated in the tables. Scatchard analyses (17) of the cortisol binding globulin and glucocorticoid receptor studies were plotted with a least squares linear regression. The correlation coefficient ( $r$ ) of the Scatchard regression line was computed with the assistance of computer programs (25, 26).

**Study of the family.** Subsequent to these studies four 24-h urines were obtained from six other family members for measurement of free cortisol.

## RESULTS

Although the circadian rhythm and episodic secretory pattern were normal in both patients (Fig. 1), the mean of 49 consecutive serum cortisol values from samples drawn every 30 min over a period of 24 h was 27.4  $\mu\text{g}/\text{dl}$  in patient I and 9.9  $\mu\text{g}/\text{dl}$  in patient II. The mean plasma cortisol in eight normal men was  $7.52 \pm 1.64$   $\mu\text{g}/\text{dl}$  (Fig. 1, Table I). The 24-h urinary-free cortisol measured on 4–5 consecutive d was also markedly increased in patient I and mildly elevated in patient II (Table I).

Cortisol binding globulin capacity and affinity were normal in both patients (Table II). However, the concentration of unbound cortisol was markedly increased in patient I and twice higher than normal in patient II (Table II).

Plasma ACTH measured at 0800 h was elevated in both patients (Table I). Serum dehydroepiandrosterone sulfate and  $\Delta^4$ -androstenedione were elevated in both father and son, more so in the father (Table I). Serum 17-hydroxyprogesterone values were within the normal range in both patients and 11-deoxycortisol was elevated in the father and normal in the son (Table I).

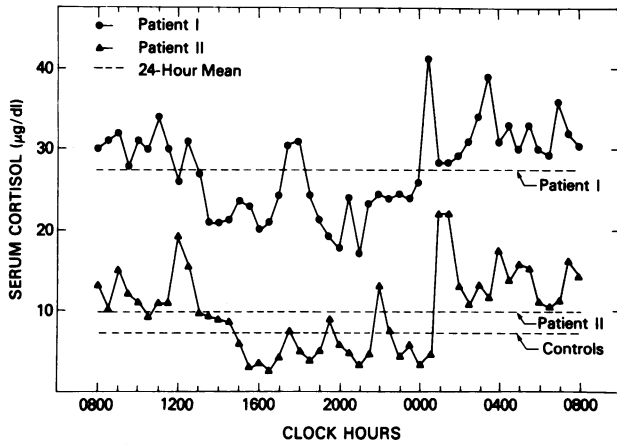


FIGURE 1 Cortisol secretion pattern over 24 h for the propositus (patient I, ●) and his son (patient II, ▲). Interrupted lines indicate the mean plasma cortisol values for all the samples drawn every 30 min over 24 h. Both patients I and II had higher 24-h mean plasma cortisol levels than the mean obtained from eight normal male controls.

The responsiveness of the hypothalamic-pituitary unit to exogenous glucocorticoids was tested with dexamethasone. Increasing doses of dexamethasone (0.3,

0.6, 1.0, 2.0, and 3.0 mg) were given orally at midnight every other day. A serum sample was drawn at 0800 h the next morning for the determination of serum cortisol and dexamethasone levels. Patient I required 3.0 mg and patient II required 1.2 mg of dexamethasone to effect a 50% suppression of the morning cortisol levels. This same degree of suppression was achieved with 0.4 mg of dexamethasone in five normal controls (Table I). To exclude the possibility of increased metabolic clearance or decreased absorption of dexamethasone in the two patients we measured concurrent plasma dexamethasone levels. The same degree of resistance to dexamethasone suppression was noted when serum cortisol values were plotted against the morning serum dexamethasone levels from the same samples (Fig. 2).

Because of the hypertension and hypokalemic alkalosis in patient I we measured 24-h urinary aldosterone, serum corticosterone, and deoxycorticosterone (Table III). Urinary aldosterone was in the low normal range in both patients. Serum corticosterone, however, was elevated in the son and markedly elevated in the father. Serum deoxycorticosterone was also increased in the father. The father's serum corticosterone de-

TABLE I  
Characteristics of Hypothalamic-Pituitary-Adrenal Function

|  | Patient I<br>(mean±SE)         | Patient II<br>(mean±SE)       | Normal<br>controls<br>(mean±SD) |
|--|--------------------------------|-------------------------------|---------------------------------|
| Mean Serum Cortisol*, µg/dl  | 27.4§                          | 9.9                           | 7.52±1.64<br>(n = 8)            |
| Dehydroepiandrosterone sulfate,<br>DHEAS, µg/dl                        | 524±88 <sup>†</sup><br>(m = 5) | 381±83<br>(m = 5)             | 284±97<br>(n = 14)              |
| Δ <sup>4</sup> -Androstenedione, ng/dl                                 | 470±86 <sup>†</sup><br>(m = 5) | 241±48<br>(m = 5)             | 198±71<br>(n = 14)              |
| 17-Hydroxyprogesterone, ng/dl  | 118±20<br>(m = 4)              | 67±11<br>(m = 4)              | 138±65<br>(n = 14)              |
| 11-Deoxycortisol, ng/dl  | 250±50 <sup>†</sup><br>(m = 4) | 80±16<br>(m = 4)              | 83±52<br>(n = 14)               |
| ACTH, pg/ml  | 155, 123                       | 175, 76                       | <75                             |
| Urinary-free cortisol, µg/24 h   | 1780±70§<br>(m = 5)            | 128±7 <sup>†</sup><br>(m = 4) | 48±22<br>(n = 22)               |
| Sensitivity to dexamethasone suppression,<br>ED <sub>50</sub> in mg/d† | 3.0§                           | 1.2§                          | 0.4±0.07<br>(n = 5)             |

\* Measured in 49 samples drawn every 30 min for 24 h.

† ED<sub>50</sub>, dose of dexamethasone required for 50% suppression of 0800 serum cortisol levels (see legend to Fig. 2).

§ P < 0.0005.

<sup>†</sup> P < 0.05.

TABLE II  
Cortisol-binding-globulin (CBG) Binding Characteristics, and Concentration of Serum Free Cortisol

|  | Patient I<br>(mean±SE) | Patient II<br>(mean±SE) | Normal<br>controls<br>(mean±SD) |
|--|------------------------|-------------------------|---------------------------------|
| CBG  |                        |                         |                                 |
| Binding capacity, $\mu\text{g}/\text{dl}$                  | 21.1±0.3<br>(m = 3)    | 17.4±1.5<br>(m = 3)     | 22.5±1.3<br>(n = 7)             |
| Binding affinity, $10^7 M^{-1}$                            | 6.0±0.5<br>(m = 3)     | 4.9±0.5<br>(m = 3)      | 5.4±0.3<br>(n = 7)              |
| Serum-free cortisol concentration, $\mu\text{g}/\text{dl}$ | 1.76±0.30*<br>(m = 4)  | 0.70±0.32<br>(m = 4)    | 0.27±0.39<br>(n = 7)            |

\* =  $P < 0.01$ .

creased to 1,370 ng/dl after a single dose of 3 mg of dexamethasone on the previous night.

Glucocorticoid receptor studies in mononuclear leukocytes or cultured skin fibroblasts revealed a normal number of receptors in both patients (Table IV). The apparent dissociation constant ( $K_d$ ) in both assay sys-

tems, however, was significantly increased in the father, implying a diminished apparent affinity of the receptor for dexamethasone (Fig. 3, Table IV). The affinity of the mononuclear leukocyte glucocorticoid receptors for dexamethasone was also low in the son (Fig. 3, Table IV). We noted no difference between the affinity of the cultured skin fibroblast glucocorticoid receptors from patient II and normal controls (Table IV).

Because a high plasma cortisol level would be expected to affect the measured  $K_d$  (27) we preincubated mononuclear leukocytes from normal controls with cortisol (100  $\mu\text{g}/\text{dl}$ ) at 37°C for 60 min before assay. There was a minor decrease in apparent affinity that could not account for the differences in glucocorticoid binding observed between the patients and normal subjects. We found that prolongation of the incubation period eliminates changes in affinity after 8-h incubation (data not shown).

The glucocorticoid receptor in the cytosol of mononuclear leukocytes from both patients showed a decrease in both affinity and concentration (Table V). In the fibroblast studies that were performed in the presence of sodium molybdate, a known glucocorticoid receptor stabilizer (28), the number of skin fibroblast cytosol receptors was low in the father but normal in the son.

All glucocorticoid receptor studies on the mononuclear leukocytes and fibroblasts from the patients were done concurrently with samples from normal control subjects. Nonspecific binding in all studies had a linear relationship with the total labeled hormone concentration and was similar in patient and control samples. Correlation coefficients of the Scatchard regression lines were  $> |0.89|$  for all assays performed. The cor-

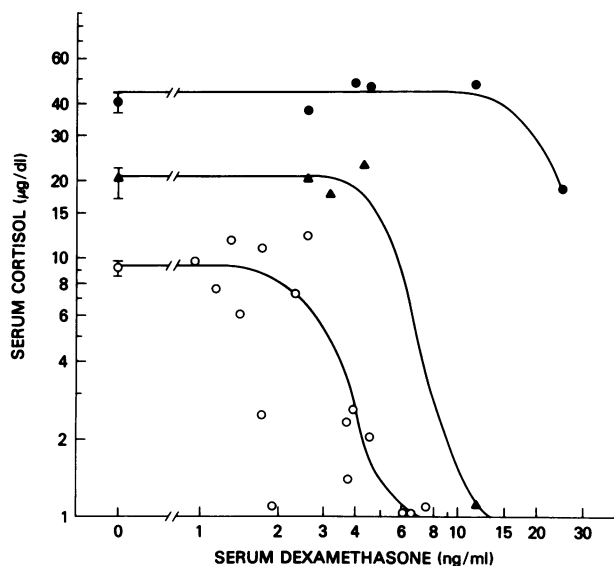


FIGURE 2 The results of a midnight dexamethasone suppression test. Dexamethasone doses ranging from 0.1 to 3 mg were given per os at midnight to the patients (patient I, ●, patient II, ▲) and five normal male controls (○). Serum cortisol and dexamethasone, measured at 8:00 a.m. the following morning, are plotted logarithmically. Higher levels of dexamethasone are required to obtain the same degree of cortisol suppression in both patients when compared with controls (Table II).

TABLE III  
Patient Mineralocorticoid Status

|  | Patient I<br>(mean±SE) | Patient II<br>(mean±SE) | Normal<br>controls<br>(mean±SD) |
|--|------------------------|-------------------------|---------------------------------|
| Urinary aldosterone, $\mu\text{g}/24\text{ h}$ | 3.4±0.3<br>(m = 5)     | 2.4±0.4<br>(m = 5)      | 4.5±2.2<br>(n = 19)             |
| Serum corticosterone, $\text{ng}/\text{dl}$    | 2870±360*<br>(m = 5)   | 1376±326*<br>(m = 6)    | 400±116<br>(n = 15)             |
| Serum DOC, $\text{ng}/\text{dl}$               | 53.9, 45.7             | 13.7, 7.2               | 0–20‡<br>n = 81                 |

\*  $P < 0.001$ .

‡ Range.

relation coefficient for glucocorticoid receptor assays on mononuclear leukocytes was  $> |0.94|$ .

One of the propositus' siblings (II<sub>10</sub>) had 24-h urinary free cortisols ranging between 140 and 213  $\mu\text{g}/24\text{ h}$  (Fig. 4). It is possible that sibling II<sub>7</sub> who had marked hypertension and died of a cerebrovascular accident at the age of 54, had the severe form of the disease.

## DISCUSSION

Both of our patients with cortisol resistance had defective glucocorticoid receptors with decreased apparent affinity for dexamethasone. The reduction in affinity correlated with the severity of the clinical pic-

ture and the plasma cortisol concentrations. Total and free plasma cortisol concentrations in the father and 24-h urinary free cortisol values in father and son were in the range seen in Cushing's syndrome. The 24-h mean plasma cortisol of the son was higher than our mean of normal men although not statistically different. The high urinary free cortisol and plasma free cortisol, however, suggest an elevation of mean 24-h plasma cortisol. Neither patient had any of the symptoms or signs of Cushing's syndrome.

The hypothalamic-pituitary-adrenal axis was resistant to dexamethasone suppression and the degree of resistance correlated with the degree of the glucocorticoid receptor affinity defect. Resistance of this axis to dexamethasone has been shown in patients with

TABLE IV  
Whole Cell Glucocorticoid Receptor Status

|   | Patient I<br>(mean±SE)    | Patient II<br>(mean±SE)   | Normal controls<br>(mean±SD) |
|---|---------------------------|---------------------------|------------------------------|
| Mononuclear Leukocytes  |                           |                           |                              |
| Binding capacity <sup>‡</sup> , $\text{fmol}/10^6\text{ cells}$ | 5.3±0.1<br>(m = 2)        | 5.6±0.1<br>(m = 2)        | 5.4±0.22<br>(n = 10)         |
| Apparent dissociation constant, $nM$                            | 7.4±1.5§<br>(m = 2)       | 3.9±0.1*<br>(m = 2)       | 2.8±0.44<br>(n = 10)         |
| Cultured Skin Fibroblasts                                       |                           |                           |                              |
| Binding capacity (R <sub>0</sub> ), $\text{sites}/\text{cell}$  | 171,300±41,700<br>(m = 3) | 121,000±30,900<br>(m = 3) | 133,400±45,300<br>(n = 6)    |
| Apparent dissociation constant, $nM$                            | 10.5±0.5*<br>(m = 3)      | 5.4±1.3<br>(m = 3)        | 6±1.3<br>(n = 6)             |

\* =  $P < 0.05$ .

§  $P < 0.0005$ .

‡ Multiply by 602 to obtain sites per cell.

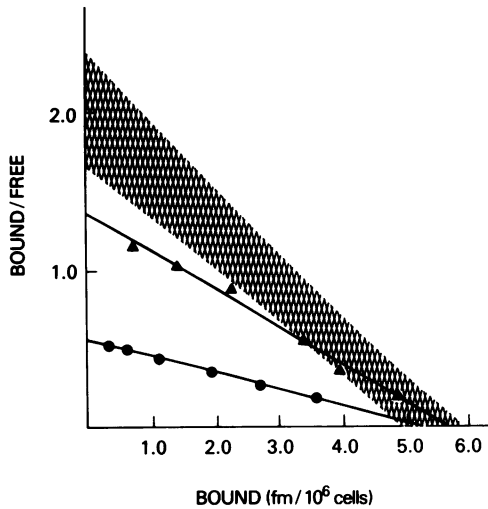


FIGURE 3 The results of the Scatchard analysis of dexamethasone binding to the glucocorticoid receptor of circulating mononuclear leukocytes in the two patients and 10 controls. The shaded area represents the normal range. The number of receptors indicated by the x-axis intercept is within the normal range. The apparent affinity indicated by the slope of the line (slope =  $-1/K_d$ ) is clearly different in patient I (●) and outside the normal range in patient II (▲).

Cushing's syndrome (29), but the glucocorticoid receptor characteristics in these patients have been reported to be normal (30). The compensatory mechanism for a defective glucocorticoid receptor affinity appears to be increased ACTH secretion to maintain plasma cortisol at levels high enough to assure adequate glucocorticoid effect. The high levels of plasma

ACTH found in these patients and the increased values of adrenal androgens in the serum support this hypothesis. All other elements of the hypothalamic-pituitary-adrenal system relating to cortisol functioned normally as shown by the pattern of plasma cortisol during the 24 h.

Both patients had elevated plasma corticosterone values and the most severely affected patient had increased plasma deoxycorticosterone concentrations as well. The increased secretion of sodium-retaining corticoids in the father was manifest clinically as hypertension and hypokalemic alkalosis. The low 24-h urinary aldosterone excretion was also consistent with this. The son did not have clinical manifestations of increased mineralocorticoid effect probably because the degree of hypercorticism observed in this patient could be sufficiently compensated by suppression of the renin-aldosterone axis. The hypersecretion of sodium-retaining corticoids in these patients was a result of increased ACTH stimulation because the increased plasma levels could be suppressed with dexamethasone.

Theoretically, the treatment of choice in the father would be dexamethasone in doses sufficient to normalize serum sodium-retaining corticoids without causing Cushing's syndrome. We elected initially to use dexamethasone 3 mg/d, because at that dose his plasma corticosterone was suppressed to the levels of the asymptomatic son.

Glucocorticoid hormones, as all the other classes of steroid hormones, exert their cellular actions by forming complexes with a specific cytoplasmic receptor, which in turn translocates to the nucleus and binds to specific sites on the chromatin (31). Receptor occu-

TABLE V  
Glucocorticoid Receptor in Cytosol

|  | Patient I<br>(mean±SE)           | Patient II<br>(mean±SE) | Normal control<br>(mean±SD) |
|--|----------------------------------|-------------------------|-----------------------------|
| Mononuclear leukocyte                    |                                  |                         |                             |
| Binding capacity, <i>fmol/mg protein</i> | 26.0±4.7<br>(-0.89) <sup>o</sup> | 53.0±8.6<br>(-0.93)     | 191.0±30<br>(-0.90)         |
| Dissociation constant, <i>nM</i>         | 3.5±1.2                          | 3.2±1.0                 | 1.7±0.7                     |
| Cultured skin fibroblasts <sup>‡</sup>   |                                  |                         |                             |
| Binding capacity, <i>fmol/mg protein</i> | 35±3.0                           | 88±27                   | 95±29.4                     |
| Dissociation constant, <i>nM</i>         | 6.0±1.5 <sup>§</sup>             | 2.8±0.3 <sup>§</sup>    | 1.4±0.34                    |

<sup>o</sup> Correlation coefficient of the Scatchard regression lines.

<sup>‡</sup> Mean from three experiments.

<sup>§</sup>  $P < 0.05$ .

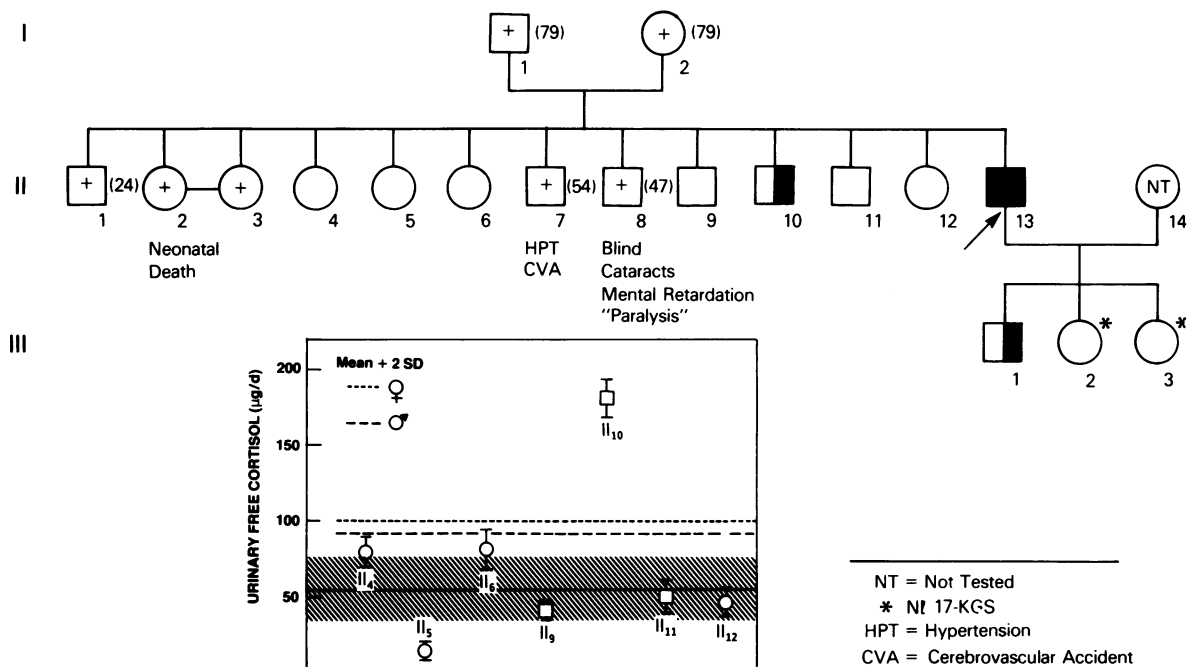


FIGURE 4 Family pedigree. Propositus II<sub>13</sub> had the severe symptomatic form of the disease (■). His son, III<sub>1</sub>, was asymptomatic but biochemically affected (◻). A brother of the propositus (II<sub>10</sub>) was also asymptomatic and had elevated urinary free cortisol (insert) at the levels of III<sub>1</sub> (◻). Another brother (II<sub>7</sub>) who had marked hypertension died of a cerebrovascular accident at the age of 54. Member II<sub>1</sub> was killed in the war. All other siblings of the propositus had normal urinary free cortisol levels. Insert: Shaded area represents the mean±SD of urinary free cortisol of normal adults in our laboratory.

pancy and therefore nuclear translocation would be decreased at normal plasma cortisol levels if receptor affinity were low.

The finding that the receptor concentration was consistently decreased only in the broken cell assays may reflect receptor instability. Because the number of receptors is maintained in the whole cell, we suggest that new receptor synthesis compensates for rapid turnover of an unstable receptor. In cell-free systems no replenishment of the receptor is possible. Further studies are in progress to delineate the exact nature of the glucocorticoid receptor defect.

Recently, a similar glucocorticoid receptor defect associated with hypercortisolemia but no stigmata of Cushing's syndrome has been found in a primate species, the squirrel monkey (32). Decreased apparent affinity in the whole cell assay in these animals is also associated with a decreased sensitivity or response to glucocorticoid hormones.

Receptor-mediated glucocorticoid hormone resistance has also been reported in mutant lymphoma cell lines in vitro (33-34). Decreased numbers of receptors or defective nuclear binding of glucocorticoid hor-

mones has been found in those instances. In the recognized instances of steroid receptor defects, the androgen receptor has been examined most closely (36). Quantitative and qualitative changes of the androgen receptor, the latter concerning thermolability or lack of stabilization with sodium molybdate have been described in syndromes of androgen resistance in man (37, 38). Progesterone end-organ resistance has been reported in a woman with a reduction in the number of endometrial progesterone receptors (39) and recent studies have shown that vitamin D-dependent rickets type II is associated with reduced nuclear uptake of the vitamin D-receptor complex in skin fibroblasts cultured from affected individuals (40). Thus, primary cortisol resistance appears to be the first human disease to be associated with defective affinity of a steroid hormone receptor (i.e., an "affinity mutant").

The genetic transmission of this disease from father to son excludes an x-linked disorder. The different clinical presentation and severity of the defect between father and son is compatible with an autosomal recessive disease presenting with a subclinical carrier state or an autosomal dominant disorder with variable



expression. We were able to study briefly other family members in an effort to understand the transmission of this disease and found only one sibling with a presumptive mild defect similar to that seen in the son. The molecular basis for possible homozygous and heterozygous states of glucocorticoid receptor affinity is presently obscure.

We should point out that if the son and his uncle represent the heterozygous state, then carriers would be suspected only if a 24-h urinary cortisol or a dexamethasone suppression test were performed. Nevertheless, the full syndrome must be quite rare because the combination of hypertension, hypokalemia, and alkalosis would initiate a search for sodium-retaining corticoids in any major medical center.

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