10. Griffith OW 1980 Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal Biochem* 106:207-212
11. Lohr GW, Waller HD 1974 In: Bergmeyer HU (ed) *Methods of Enzymatic Analysis*, Vol 2. Academic New York, pp 636-643
12. Ysebaert-Vanneste M, Vanneste WH 1980 Quantitative resolution of Cu, Zn- and Mn-superoxide dismutase activities. *Anal Biochem* 107:86-95
13. Carlberg I, Altmejd B, Mannervik B 1981 Purification and immunological studies of glutathione reductase from rat liver. *Biochim Biophys Acta* 677:146-152
14. Paglia DE, Valentine WN 1967 Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 70:158-169
15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ 1951 Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265-275
16. Kimbell RE, Reddy K, Peirce, TH, Schwartz LW, Mustafa MG, Cross CE 1976 Oxygen toxicity: augmentation of antioxidant defense mechanisms in rat lung. *Am J Physiol* 230:1425-1431
17. Nishiki K, Jamieson D, Oshino N, Chance B 1976 Oxygen toxicity in the perfused rat liver and lung under hyperbaric conditions. *Biochem J* 160:343-355
18. Bucher J, Roberts R 1981 The development of the newborn rat lung in hyperoxia: a dose-response study of lung growth maturation, and change in antioxidant enzymes activities. *Pediatr Res* 15:999-1008
19. Frank L, Bucher JR, Roberts RJ 1978 Oxygen toxicity in neonatal and adult animals of various species. *J Appl Physiol* 45:699-704
20. Yam J, Frank L, Roberts RJ 1978 Oxygen toxicity: comparison of lung biochemical responses in neonatal and adult rats. *Pediatr Res* 12:115-119
21. Kehrer JP, Auer AP 1977 Age-dependent lipid peroxidation in neonatal rat lung tissue. *Arch Biochem Biophys* 181:73-81
22. Orrenius S, Ormstad K, Thor H, Jewell SA 1983 Turnover and functions of glutathione studied with isolated hepatic and renal cells. *Fed Proc* 42:3177-3188
23. Lauterburg BH, Smith CV, Hughes H, Mitchell J 1984 Biliary excretion of glutathione and glutathione disulfide in the rat: regulation and response to oxidative stress. *J Clin Invest* 73:124-133
24. Yam J, Frank L, Roberts RJ 1978 Age-related development of pulmonary antioxidant enzymes in the rat. *Proc Soc Exp Biol Med* 157:293-296
25. Kimura RE, Thulin GE, Wender D, Warshaw JB 1983 Decreased oxidative metabolism in neonatal rat lung exposed to hyperoxia. *J Appl Physiol* 55:1501-1505
26. Warshaw JB, Rosenthal MD 1972 Changes in glucose oxidation during growth of embryonic heart cells in culture. *J Cell Biol* 52:283-291

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Modulation of Glucocorticoid Secretion by Growth Hormone

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ABSTRACT. We measured the cortisol and corticosterone responses to insulin-induced hypoglycemia in 13 growth hormone (GH)-deficient children and 30 short children without GH deficiency. Although there was no difference between the two groups in 1) degree of hypoglycemia attained, 2) baseline cortisol, 3) baseline corticosterone, or 4) cortisol 40 min after insulin injection, GH-deficient children had a significantly greater corticosterone response to this stress (3.6 ± 0.4 versus 1.9 ± 0.2 $\mu\text{g/dl}$). (All data are presented as mean $\pm$ SEM.) In order to explore the effect of GH on corticosterone secretion, we measured cortisol and corticosterone responses to synthetic (1-24) ACTH before and after 3 days of exogenous GH (0.2 unit/kg/day). In 13 GH-deficient children, GH treatment caused a significant decrease in the corticosterone response to ACTH (2.2 ± 0.2 $\mu\text{g/dl}$ before GH to 1.6 ± 0.2 $\mu\text{g/dl}$; $t = 5.22$, $p < 0.001$; paired t test) despite the fact that there was no significant change in the cortisol response to ACTH (18 ± 2 $\mu\text{g/dl}$ before and 16 ± 2 $\mu\text{g/dl}$ after). When seven short children who were not GH deficient underwent a

similar 3-day course of GH, the decrease in their corticosterone response was much less although still statistically significant (2.0 ± 0.5 to 1.8 ± 0.5 $\mu\text{g/dl}$; paired t test, $p < 0.05$). Again, the stimulated levels of cortisol were not affected by GH treatment (19 ± 4 versus 18 ± 3 $\mu\text{g/dl}$). These results indicate that GH modulates the adrenal response to ACTH by suppressing corticosterone secretion without affecting cortisol secretion. In summary, this study presents two new findings. First, corticosterone levels are elevated during insulin tolerance testing in children with GH deficiency and, second, a 3-day course of GH replacement therapy causes a reduction in corticosterone serum levels after ACTH administration to more normal levels without altering the corresponding cortisol concentrations. As corticosterone is a less potent glucocorticoid, it might function as a cortisol antagonist when present in increased amounts and play a role in the poor recovery from hypoglycemia frequently observed in individuals with GH deficiency. (*Pediatr Res* 19: 823-827, 1985)

Abbreviations

GH, growth hormone
ACTH, adrenocorticotrophic hormone
RIA, radioimmunoassay
SmC, somatomedin C

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Glucocorticoid secretion and function has been extensively studied in man and animal model systems. Cortisol and/or corticosterone are the main glucocorticoids secreted by the adrenal cortex in response to ACTH and they act in the liver to cause an increase in blood glucose levels. The latter process is mediated, at least in part, by binding to specific receptors, translocation to the nucleus, and subsequent synthesis of specific proteins. Generally, without further consideration, rats have been used to develop models for the specific steps in the process. However, the nature of the glucocorticoids in man is very different from that in rats. Rats synthesize only corticosterone whereas humans produce both corticosterone (2 mg/day) and cortisol (20 mg/day) (1). This difference may be significant because changes in glucose homeostasis and salt balance could be affected by the relative amounts of the two compounds as they do not have equal potency for both functions in bioassays (2). In humans, but not in rats, both are secreted in response to ACTH, and both show large variation in serum concentration depending on the temporal relationship to the last episode of ACTH secretion (3). ACTH acts to increase glucocorticoid secretion by increasing the concentration of cyclic AMP, the second messenger (4). In turn, cyclic AMP increases conversion of cholesterol to pregnenolone (5). Regulation of the relative amounts of cortisol *versus* corticosterone cannot be mediated solely by ACTH and cyclic AMP because pregnenolone is a precursor of both glucocorticoids and an increase in pregnenolone synthesis ought to lead to proportional increases in both cortisol and corticosterone. Consequently, in order to change the relative proportions of these compounds, a second hormone must be involved. In this study, we show that GH-deficient children produce more corticosterone in response to insulin-induced hypoglycemia than do normal children even though there is no significant difference in cortisol secretion. The increase can be reduced after a short course of growth hormone therapy. Together these observations suggest that GH modulates adrenal cortex responses to ACTH at a step in the pathway after synthesis of pregnenolone, perhaps by regulating the amount of 17-hydroxylase activity.

MATERIALS AND METHODS

Subjects. The responses of cortisol and corticosterone to insulin-induced hypoglycemia were studied in 13 children (three girls, 10 boys) with GH deficiency. The 12 children with idiopathic GH deficiency (ages 4.25–14.42 yr) were all prepubertal. One 11.5-yr-old boy with GH deficiency secondary to septo-optic dysplasia was in midpuberty. GH deficiency was considered to be present if the child failed to raise his serum GH level above 10 ng/ml in response to both insulin-induced hypoglycemia and a combined arginine/L-dopa tolerance test.

Thirty children (20 boys, 10 girls) with normal growth rates and a GH response of more than 10 ng/ml to insulin-induced hypoglycemia or combined L-arginine/L-dopa stimulation served as controls. Hypoglycemia in response to an intravenous bolus of insulin (0.1 unit/kg) was considered to be adequate if serum glucose decreased by more than 50% from the fasting value.

ACTH stimulation tests were done after overnight dexamethasone suppression (1 mg/m², po at 2200) (7, 8). An intravenous line was inserted between 0730 and 0830 and one or two (depending on the size of the child) baseline samples were drawn over a 15-min interval. An intravenous bolus of synthetic 1-24 ACTH (0.25 mg, Cortrosyn) was injected at time 0 and samples for cortisol and corticosterone were taken 30 and 45 min afterward. Ten children with isolated GH deficiency (AM cortisol of greater than 20 µg/dl) and two with combined GH and partial ACTH deficiency (maximum cortisol following insulin <8 µg/dl, failure to increase 11-desoxycortisol after metyrapone) were studied before and after 3 days of GH treatment (0.1 unit/kg/ every 12 h, intramuscular). An additional three GH-deficient children were studied 48 h after the start of GH therapy, and one GH-deficient child was studied 24 h after the start of GH

therapy. For these four children the initiation of GH therapy was delayed in order to explore the time course of the changes in corticosterone secretion. In all cases there was a 72-h interval between ACTH stimulation tests, and the second ACTH test took place 12 h after the last GH injection. Seven non-GH-deficient children (four with delayed puberty, two with short stature without a delayed bone age, and one with Aarskog's syndrome) received GH according to the same protocol and served as controls. Cortisol and corticosterone responses to a single ACTH bolus were also studied in 11 healthy children of normal height. All subjects were euthyroid when studied.

Assays. Cortisol was measured by RIA with kits purchased from Amersham (Chicago, IL). Corticosterone was measured by RIA using a rabbit antibody prepared in our laboratory against 11β,21-dihydroxy-4-pregnen-3,20-dione-3-carboxymethoxyoxime: bovine serum albumin conjugate (Steraloids, Wilton, NH). In this assay 0.3 ml of serum, 2.5 nCi of [1,2,6,7-³H]corticosterone [purchased from New England Nuclear, Boston, MA and purified by chromatography on Sephadex LH-20 (9)] and 0.6 ml of ethyl acetate were mixed and allowed to stand at room temperature for 10 min. An aliquot (100 µl) of the ethyl acetate layer was removed and counted in a β-counter in order to estimate recovery following extraction. Two aliquots of 100 µl each were dried under a stream of nitrogen prior to RIA. The assay mixture contained [1,2,6,7-³H]corticosterone (10 nCi/tube), assay buffer (0.5% BSA, 50 mM Na₂HPO₄, 15 mM sodium azide, pH 7.4), and antibody at a final dilution of 1:667 in a total volume of 300 µl. After an overnight incubation at 4°C, 250 µl of Dextran-coated charcoal (5 g/liter of Norit A, 0.5 g/liter Dextran T-70, and 4.5 g/liter NaCl in assay buffer) were added to separate free from antibody-bound corticosterone. Recovery of added tracer was 86 ± 5%; intrassay variation was 8%, and interassay variability was 10%. The limit of detectability of corticosterone was 20 pg which would be equivalent to 0.08 µg/dl. Cortisolone, 17-hydroxyprogesterone, and androstenedione all had less than 0.1% cross-reactivity. Cortisol showed 0.2% cross-reactivity; progesterone had 2.0% cross-reactivity; desoxycorticosterone had 26% cross-reactivity.

The validity of this assay procedure for corticosterone in human serum was confirmed by chromatography on a Sephadex LH-20 column packed in a Pharmacia SR-10J column (1 × 40 cm) with the solvent system benzene-methanol (4:1). The column was eluted in an upward flow mode with a Milton-Roy mini-pump at a flow rate of 60 ml/h. After addition of tracer, serum samples (0.6 ml) were extracted with ethyl acetate (1.2 ml) and aliquots assayed directly for corticosterone. An additional 0.3 ml of the extract was evaporated to dryness, dissolved in 0.4 ml of benzene-methanol (4:1), and chromatographed. Fractions of 2 ml were collected and assayed for radiolabel (0.5 ml) and corticosterone (1.0 ml). When corticosterone was added to corticosterone-free serum, all of the recovered corticosterone cochromatographed with the authentic labeled steroid. Correcting for the losses inherent in the size of each fraction and aliquot, the recovery was essentially quantitative (>90%) when the added amount of corticosterone was from 0.3 to 5 µg/dl. When sequential samples from an ACTH stimulation test from a normal adult were treated in the same way, the major cross-reacting peak cochromatographed with authentic labeled corticosterone. Although one other minor peak (less than 10%) was detected, there was good correspondence (within 10%) between the corticosterone measured in the column eluate and the corticosterone cross-reacting material in the whole extract. This chromatographic procedure was repeated with samples from an ACTH test in a GH-deficient child and with two sets of samples from the insulin tolerance test with both normal and GH-deficient children. The corticosterone values observed in the chromatographed extract and the total extract agreed (±10%) in all cases. Therefore, all of the data presented here were obtained without chromatography of the serum extract.

SmC was assayed by RIA (10) after acid-ethanol extraction

(11) using antibody supplied by the National Hormone and Pituitary Program.

Statistics. Student's *t* test and Wilcoxon's rank sum test were used to determine the statistical significance of differences between population means. The paired *t* test was used to determine the significance of changes before and after GH. Values are reported as the mean $\pm$ SEM (12).

RESULTS

Figure 1 shows the time course of glucocorticoid secretion for two children, one with normal GH levels and one with GH deficiency. The sequence of events after insulin administration at the start of the test is (at about 20-min intervals) 1) most intensive hypoglycemia, 2) highest glucocorticoid levels, and 3) GH secretion. As the GH secretory episode occurs at the same time as or shortly after the glucocorticoid secretory episode, GH cannot directly stimulate or inhibit corticosterone secretion during this test.

The glucose, cortisol, and corticosterone responses to insulin-induced hypoglycemia in GH-deficient and control children are summarized in Table 1. There was no significant difference between GH-deficient children and controls with respect to baseline cortisol, cortisol after insulin, glucose nadir, or baseline corticosterone. However, GH-deficient children had significantly higher corticosterone levels 40 min after the insulin injection. As expected, there was a significant difference in serum SmC levels between the two groups.

After 3 days of GH therapy, SmC levels increased from 0.31

± 0.09 to 1.11 ± 0.23 unit/ml ($p < 0.02$, paired *t* test) in the GH-deficient children. The children without GH deficiency also had an increase in SmC from 1.09 ± 0.38 to 2.81 ± 0.53 unit/ml ($p < 0.01$).

The effect of a 3-day course of GH treatment on corticosterone secretion in response to an ACTH bolus is shown in Figure 2. For comparative purposes the responses of children of normal stature to ACTH are also shown (C). Thus, the increased corticosterone response to endogenous ACTH (released in response to hypoglycemia) was also observed in response to exogenous ACTH. When the two children with ACTH deficiency (and thus decreased cortisol and corticosterone secretion) are excluded from the calculation, the mean corticosterone response to exogenous ACTH was 2.42 ± 0.22 μ g/dl for GH-deficient children prior to treatment versus 1.53 ± 0.19 μ g/dl for children of normal stature ($p < 0.01$ by both Student's *t* test and Wilcoxon rank sum test). In the short children that comprised our non-GH deficient group (B), the corticosterone levels after ACTH administration were 2.0 ± 0.5 μ g/dl. However, one of these children had extremely high corticosterone (5 μ g/dl) levels in response to ACTH. This child was dysmorphic and extremely short, had a very delayed bone age, and had a severely disturbed home environment but was not GH deficient and did not respond to GH therapy; we could not determine the exact cause of her short stature or the cause of the high serum corticosterone levels secreted in response to ACTH.

GH treatment did not change the suppressed baseline cortisol (< 1.0 μ g/dl) and corticosterone (< 0.1 μ g/dl) levels. The cortisol level after stimulation was unaffected by GH treatment (18 ± 3

Fig. 1. Response to insulin-induced hypoglycemia in a child with GH deficiency (A) and a short child without GH deficiency (B). At time zero each subject received 0.1 unit/kg of regular insulin as an intravenous bolus. Serum samples were taken at the indicated times. Glucose (○—○); GH (■—■); cortisol (●—●); corticosterone (□—□).

Table 1. Response to insulin-induced hypoglycemia

Patient	Sm-C (unit/ml)	Glucose nadir (mg/dl)	Cortisol (baseline) (μ g/dl)	Corticosterone (baseline) (μ g/dl)	Cortisol (stim)* (μ g/dl)	Corticosterone (stim) (μ g/dl)
Normal ($n = 30$)	$0.86 \pm 0.07^\dagger$	36 ± 2	23 ± 1	1.6 ± 0.2	23 ± 1	1.9 ± 0.2
GH deficient ($n = 13$)	$0.22 \pm 0.05^\ddagger$	31 ± 3	19 ± 1	1.7 ± 0.3	25 ± 2	$3.6 \pm 0.4^\ddagger$

* Stimulated cortisol and corticosterone levels were measured on serum obtained forty minutes after the administration of insulin.

† Values are reported as mean $\pm$ SEM.

‡ $p < 0.001$ compared to normal.

Fig. 2. Effect of GH on the corticosterone secretory response to ACTH after dexamethasone suppression. Each of the values shown is the average of the samples taken 30 and 45 min after administration of ACTH. A shows the result in children with isolated GH deficiency (○—○) and combined GH and ACTH deficiency (△—△). B shows the results in short children without GH deficiency. C shows the corticosterone secretory response to a single injection of ACTH after dexamethasone suppression in 11 children of normal stature.

$\mu\text{g/dl}$ before and $16 \pm 2 \mu\text{g/dl}$ after 3 days of GH). In the non-GH-deficient children, GH treatment also had no significant effect on the stimulated cortisol response to ACTH ($19 \pm \mu\text{g/dl}$ before GH and $18 \pm 4 \mu\text{g/dl}$ after GH).

In the children with GH deficiency, there was a significant (2.2 ± 0.2 versus $1.6 \pm 0.2 \mu\text{g/dl}$; $t = 5.22$, $p < 0.001$) decrease in the corticosterone level after ACTH stimulation after 3 days of GH treatment. This decrease was seen both in children with normal ACTH reserves and in the two children with evidence of ACTH deficiency. In the non-GH-deficient children, the corticosterone response to ACTH showed a statistically significant decrease ($t = 2.73$, $p < 0.05$), but the magnitude of the decrease was much smaller than in the children with GH deficiency (0.56 ± 0.11 versus $0.12 \pm 0.05 \mu\text{g/dl}$, $t = 3.70$, $p < 0.01$).

In the three GH-deficient children who received GH for 48 h prior to the second ACTH test, there was still a significant decrease in the maximal corticosterone response (1.7 ± 0.4 to $1.3 \pm 0.5 \mu\text{g/dl}$, $t = 10.0$, $p < 0.01$), and a significant increase in SmC (0.43 ± 0.46 to 1.41 ± 0.6 unit/ml; $t = 4.51$, $p < 0.05$). A single child who received GH for 24 h prior to testing also showed a decrease in his corticosterone response (1.8 to $1.6 \mu\text{g/dl}$).

DISCUSSION

In this report we present two new findings. First, corticosterone secretion is elevated in response both to endogenous and exogenous ACTH in children with GH deficiency. Second, a 3-day course of GH replacement therapy causes a significant decrease in corticosterone secretion (toward the normal levels) without altering cortisol secretion. Although GH receptors are present in high concentrations in the adrenal cortex (13) and there is an accumulation of labeled GH in the adrenal when [^{125}I] GH is administered to rats (14), a specific regulatory effect of GH on this tissue has not been previously recognized. Thus, these experiments demonstrate that GH has a significant effect on steroid production in the adrenal cortex.

This study was prompted by the observation that children with GH deficiency often have poorer recovery from insulin-induced

hypoglycemia than do children without GH deficiency. The poor recovery suggested a defect in a counter regulatory hormone such as a glucocorticoid. In fact, cortisol measurements during the test were frequently made to evaluate simultaneously pituitary reserves of ACTH and the response of the adrenal cortex. Because both cortisol and corticosterone are produced in relatively large amounts in humans, it seemed appropriate to measure both compounds. As shown in Table 1, although there was no statistically significant difference in the other parameters we measured, there was almost a 2-fold greater increase in corticosterone secretion in children with GH deficiency when compared to normal children. This effect could be caused either by a difference in the secretion of the pituitary trophic hormone or by a difference in the effect of the trophic hormone on the adrenal cortex itself. To distinguish these possibilities we developed a protocol for repeated ACTH administration. If the corticosterone secretion was normal after stimulation by exogenous ACTH, then the difference must be at the pituitary level; if the corticosterone secretion was exaggerated, then the difference must be in the adrenal cortex itself.

Previously, it has been shown that when ACTH is administered on sequential days, the amount of cortisol secreted in response to the second ACTH bolus is increased. However, when the second test is delayed for at least 3 days, there is no such increase (15). In order to avoid the effect of repeated injections of ACTH on adrenal secretion, we performed the ACTH stimulation tests 3 days apart. We found a substantial decrease in corticosterone following GH treatment although there was no effect on serum cortisol levels. The magnitude of the change in corticosterone levels in GH-deficient children was three times larger than the change seen in normal children on the same protocol. Thus, GH modulates the production of individual glucocorticoids in the adrenal cortex itself. Since the difference in structure between cortisol and corticosterone is only the presence of an hydroxy group at the 17 position, GH apparently modulates glucocorticoid production by regulating the amount of 17-hydroxylase activity.

Most studies of the effect of GH on adrenal function have

used the rat as a model. In intact rats GH treatment results in an increase in corticosterone synthesis probably due to an increase in mitochondrial cholesterol side-chain cleavage activity (16, 17). In hypophysectomized rats, combined replacement of GH and ACTH results in an increased trophic effect compared to that seen with ACTH alone. There is an increased activity of all of the microsomal hydroxylases, but it is difficult to separate the effects of GH in this system from those of ACTH itself (18). Thus, in both of these experiments with rats, corticosterone secretion increased in response to GH administration and this is the exact opposite of our observation in humans. However, there are two significant differences between our studies in humans and the studies with rats. First, a rat model with isolated GH deficiency does not exist. Thus, the first experiment evaluated pharmacological doses of GH and the second experiment attempted to evaluate combined deficiency of all hormones secreted by the anterior pituitary. Second, rats use only corticosterone as a glucocorticoid and do not synthesize cortisol whereas in humans, cortisol is the major glucocorticoid and corticosterone is produced in much smaller amounts. In fact since the apparent target of GH action in the human adrenal is the 17-hydroxylase enzyme activity and since that enzyme activity is not used by the rat to produce glucocorticoids, GH cannot be acting in the rat adrenal cortex in the same manner and, consequently, the rat is not a good model for the process.

The increase in corticosterone secretion during chronic GH deficiency, which would be a logical consequence of our experiments, may be a factor in the hypoglycemia frequently seen in GH-deficient children. Although both cortisol and corticosterone are bound to the glucocorticoid-binding protein, the protein has a greater affinity for cortisol (19). Thus, a greater amount of corticosterone would be unbound and available to the receptor for glucocorticoids. However, corticosterone is a weaker agonist for glucocorticoid activity than is cortisol and may act as an antagonist when present in excess (20). In addition to a direct effect at the receptors for glucocorticoids in the liver, corticosterone might also serve as an antagonist in the adrenal medulla. The secretion of catecholamines is sharply decreased in children with GH deficiency (21). Rudman *et al.* (22) showed that although the secretion of norepinephrine was unchanged in these children, the synthesis of epinephrine was decreased 80–90%. The enzyme that converts norepinephrine to epinephrine (phenylethanolamine N-methyl transferase) requires a high concentration of a glucocorticoid for activity (23). Since corticosterone is less effective as an agonist, it might serve as a partial antagonist in the medulla and not activate this enzyme. Thus, there might be a dual mechanism for the delay in the recovery process from episodes of hypoglycemia.

REFERENCES

- Peterson RE 1957 Plasma corticosterone and hydrocortisone levels in man. *J Clin Endocrinol Metab* 17:1150–1157
- Axelrad BJ, Cates JE, Johnson BB, Luetscher JA 1954 Bioassay of mineralocorticoids: relationship of structure to physiological activity. *Endocrinology* 55:568–574
- Nabors CJ, West CD, Mahajan DK, Tyler FH 1974 Radioimmunoassay of human plasma corticosterone: method, measurement of episodic secretion and adrenal suppression and stimulation. *Steroids* 23:363–378
- Hayashi K, Sala G, Catt KJ, Dufau ML 1979 Regulation of steroidogenesis by adrenocorticotrophic hormone in isolated adrenal cells. *J Biol Chem* 254:6678–6683
- Gill GN 1979 ACTH regulation of the adrenal cortex. In: Gill GN (ed) *Pharmacology of Adrenal Cortical hormones*. Pergamon Press, Oxford, pp 35–36
- Weldon VV, Gupta SK, Klingensmith G, Clarke WL, Duck SC, Haymond MW, Pagliara AS 1975 Evaluation of growth hormone release in children using arginine and L-dopa in combination. *J Pediatr* 87:540–544
- Child DF, Bullock DE, Hillier VF, Anderson DC 1979 Heterogeneity in adrenal steroidogenesis in normal men and women. *Clin Endocrinol (Oxf)* 11:383–389
- Granoff AB, Chasalow FI, Blethen SL 1985 17-Hydroxyprogesterone response to ACTH in children with premature adrenarche. *J Clin Endocrinol Metab* 60:409–415
- Chasalow FI 1979 Mechanism and control of rat testicular steroidogenesis. *J Biol Chem* 254:3000–3005
- Furlanetto RW, Underwood LE, Van Wyk JJ, D'Ercole AJ 1977 Estimation of somatomedin-C levels in normals and patients with pituitary disease by radioimmunoassay. *J Clin Invest* 60:648–657
- Daughaday WH, Mariz IK, Blethen SL 1980 Inhibition of access of bound somatomedin to membrane receptor and immunobinding sites: a comparison of radioreceptor and radioimmunoassay of somatomedin in native and acid-ethanol-extracted serum. *J Clin Endocrinol Metab* 15: 781–788
- Swinscow TDV 1981 *Statistics at Square One*. British Medical Association, London
- Posner BI, Kelly PA, Shiu PC, Friesen HG 1974 Studies of insulin, growth hormone and prolactin binding: tissue distribution, species variation and characterization. *Endocrinology* 95:521–531
- DeKrester DM, Catt KJ, Burger HG, Smith GC 1969 Radioautographic studies on the localization of ^{125}I -labeled human luteinizing and growth hormone in immature male rats. *J Endocrinol* 43:105–111
- Kolanowski J, Pizarro MA, Crabbe 1975 Potentiation of adrenocortical response upon intermittent stimulation with corticotropin in normal subjects. *J Clin Endocrinol Metab* 41:453–465
- Coyne MD 1984 Effect of growth hormone and corticotropin on steroidogenesis in cultured rat adrenocortical cells. *Horm Res* 19:185–190
- Kramer RE, Greiner JW, Colby HD 1977 Site of action of growth hormone on adrenocortical steroidogenesis in rats. *Endocrinology* 101:297–303
- Murakami N, Takahashi K 1983 Enhancement of corticosterone release by repeated injections of ACTH in the dexamethasone pre-treated rat. *Acta Endocrinol* 102:583–588
- Daughaday WH 1958 Binding of corticosteroids by plasma proteins III. The binding of corticosteroid and related hormones by human plasma and plasma protein fractions as measured by equilibrium dialysis. *J Clin Invest* 37:511–518
- Brorson I 1968 Concentration of corticosterone and cortisol in peripheral plasma of patients with adrenocortical hyperplasia and normal subjects. *Acta Endocrinol* 58:445–462
- Voorhess ML, MacGillivray MH 1984 Low plasma norepinephrine responses to acute hypoglycemia in children with isolated growth hormone deficiency. *J Clin Endocrinol Metab* 59:790–793
- Rudman D, Moffitt SD, Fernhoff PM, Blackston RD, Faraj BA 1981 Epinephrine deficiency in hypocorticotrophic hypopituitary children. *J Clin Endocrinol Metab* 53:722–729
- Wurtman RJ, Axelrod J 1966 Control of enzymatic synthesis of adrenaline in the adrenal medulla by adrenal cortical steroids. *J Biol Chem* 241:2301–2305