Mechanism of Estrogenic Action in Acromegaly

ERNEST SCHWARTZ, ELSA ECHEMENDIA, MARTIN SCHIFFER, and VINCENT A. PANARIELLO

From the Metabolic Unit, Veterans Administration Hospital, Bronx, New York 10468, and the Department of Medicine, Cornell University Medical College, New York

ABSTRACT In four acromegalic patients, estrogen therapy did not significantly alter the mean values of basal radioimmunoassayable plasma growth hormone. In two patients, estrogen therapy did not qualitatively alter the lack of reduction of plasma growth hormone levels after oral administration of glucose, nor did it reduce in these patients the response of plasma growth hormone to insulin-induced hypoglycemia. In one of the patients, insulin sensitivity with respect to glucose and the hypoglycemia-induced growth hormone rise seemed greater during estrogen therapy. Despite the absence of demonstrable reductions in plasma growth hormone level under varying experimental circumstances, the administration of estrogen resulted in reduction of urinary calcium and hydroxyproline excretion, in reduction of radiocalcium bone accretion rates and exchangeable pools, in reduction of serum phosphorus, and in more negative nitrogen balances. The experimental data therefore suggest that estrogen may be a peripheral antagonist of the effects of excessive growth hormone secretion in acromegaly.

INTRODUCTION

Estrogenic therapy of acromegalic patients has been shown to result in clinical benefit (1), decrease of elevated serum phosphorus levels and of urinary calcium excretion (2), and amelioration of glucose intolerance (3, 4). However, in four of five patients thus far reported, administration of estrogen did not reduce elevated plasma levels of radioimmunoassayable growth hormone (4, 5). The present studies have attempted to define the effect of estrogen therapy upon four male acromegalic patients. None of the patients showed significant decline of elevated basal plasma growth hormone levels, but all patients manifested a return towards normal of abnormal chemical and radiocalcium kinetic parameters.

Preliminary reports of this work were published in abstract form: *J. Clin. Invest.* **46:** 1115 and presented at the International Symposium on Growth Hormone, Milan, Italy, September 1967.

Received for publication 10 June 1968 and in revised form 20 September 1968.

METHODS

Clinical data and protocol. The subjects were four male acromegalic patients with elevated plasma growth hormone levels and normal thyroidal, adrenal, and gonadal function. Subject S.P., age 45, had the onset of acromegaly at age 24, and at age 36 of diabetes mellitus treated with NPH insulin,[1] 45–100 U daily. At age 36, he was given three separate courses of radiotherapy to the pituitary gland, with a dosage totaling 7000 R. Subject G.H., age 43, had the onset of acromegaly at age 40 and had received no therapy. Subject M.L., age 44, had the onset of acromegaly at age 20, and had two courses of radiotherapy to the pituitary at age 33 and at age 40 totaling 7000 R. He had a subtotal tryroidectomy for a colloid adenoma at the age of 36, and subtotal gastrectomy for bleeding duodenal ulcer at the age of 41. Subject A.R., age 47, had the onset of acromegaly at age 26, and, at the age of 44, a course of radiotherapy to the pituitary gland totaling 4000 R. He had a subtotal thyroidectomy for a colloid adenoma at the age of 37, and passed a left renal calculus at the age of 46.

Before and during estrogen therapy, the four patients underwent various combinations of complete or partial metabolic balance studies (all patients), serial plasma growth hormone radioimmunoassay determinations (all patients), radiocalcium kinetic studies (three patients), and insulin and glucose tolerance tests (two patients). Patients G.H. and M.L. were on continuous metabolic balance regimens throughout their studies, except for a 3-day interval during which patient G.H. consumed a supervised diet as an outpatient. Patients S.P. and A.R. each underwent short-term formal balance studies and radiocalcium kinetic studies before and after therapy with estrogen. During the intervals between the metabolic balance studies, each patient consumed a supervised diet of approximately constant calcium intake and continued to collect 24-hr urine specimens. Patient S.P. was in the hospital throughout his period of observation, and patient A.R. was an outpatient when not on a metabolic balance regimen.

Metabolic balance and radiocalcium methods. Previous publications have described in detail the metabolic balance procedures and radiocalcium kinetic procedures used in our

[1] NPH insulin, neutral protamine Hagedorn insulin.

TABLE I

*Growth Hormone Levels and Radiokinetic Data**

Patient, date of isotope injection, regimen	Mean Plasma growth hormone	Accretion rate and exchangeable pools				Urinary excretion			Fecal excretion		Total isotope excretion	Absorption ($I-I_{un}$)
						Urinary Ca		Urinary isotope excretion	Endogenous fecal Ca	Fecal isotope excretion		
		Time calculated	A	E	E_{2h}	Measured	Calculated					I
	($m\mu g/ml$)	(*days*)	(*g/day*)	(*g*)	(*g*)	(*g/day*)		*% dose*	(*g/day*)	*% dose*	*% dose*	*%*
S. P.	88	7	0.85	11.8	3.1	0.39	0.39	20.2	0.34	7.1	27.3	68
3-6-63	(1)‡	15						26.0		10.9	36.9	
^{47}Ca 27 μc Control												
9-20-63	86	7	0.52	8.4	3.5	0.14	0.14	8.2	0.23	10.0	18.2	39
^{47}Ca 15 μc	(4)	15						14.0		17.6	31.6	
Estinyl 6 months												
M. L.	12	7	1.41	10.2	3.8	0.34	0.34	14.1	0.19	7.1	21.2	52
10-18-66	(9)	16						18.3		10.2	28.5	
^{47}Ca 15 μc Control												
12-7-66	18	7	1.03	9.04	3.2	0.14	0.13	6.8	0.18	10.2	17.0	53
^{47}Ca 15 μc	(19)	16						9.6		14.0	23.6	
Estinyl 1 month												
A. R.	102	7	1.69	10.4	3.6	0.33	0.34	12.9	0.20	5.0	17.9	71
10-14-66	(4)											
^{47}Ca 15 μc Control												
12-12-66	103	7	1.28	9.7	3.5	0.11	0.12	5.0	0.20	8.0	13.0	41
^{47}Ca 8.3 μc	(11)											
Estinyl 42 days												

* A = accretion rate; E = exchangeable calcium; E_{2h} = 2 hr calcium pool after rapid injection; $\frac{I-I_{un}}{I} = \frac{\text{(intake—unabsorbed intake)}}{\text{intake}}$ where I_{un} = fecal Ca-endogenous fecal Ca.

‡ No. of determinations averaged.

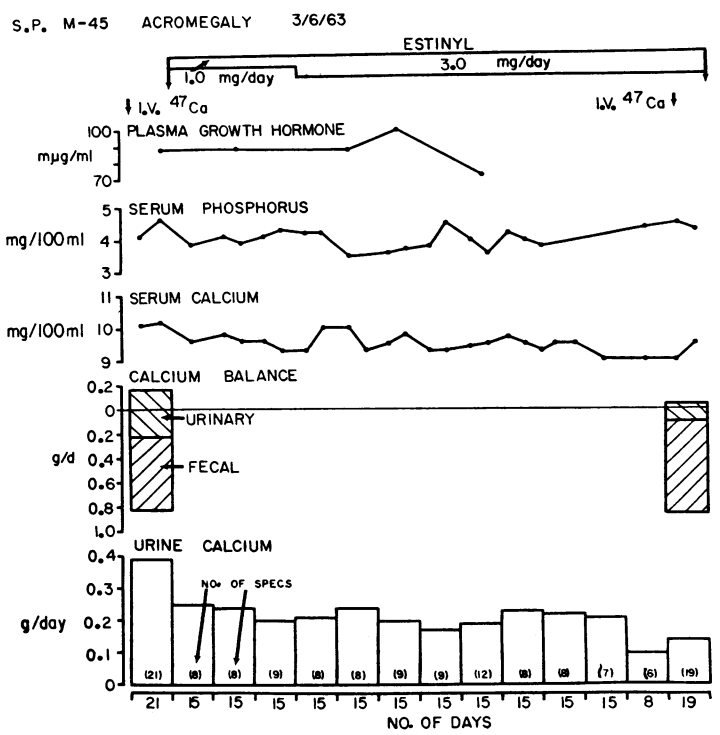

FIGURE 1 Metabolic data in patient S.P., a 45 yr old acromegalic male. Ethinyl estradiol (Estinyl) therapy in dosages up to 3.0 mg/day effected a reduction of urinary calcium excretion and less negative calcium balance. Plasma growth hormone values were obtained during the first half of the estrogen therapy period and remained relatively unchanged.

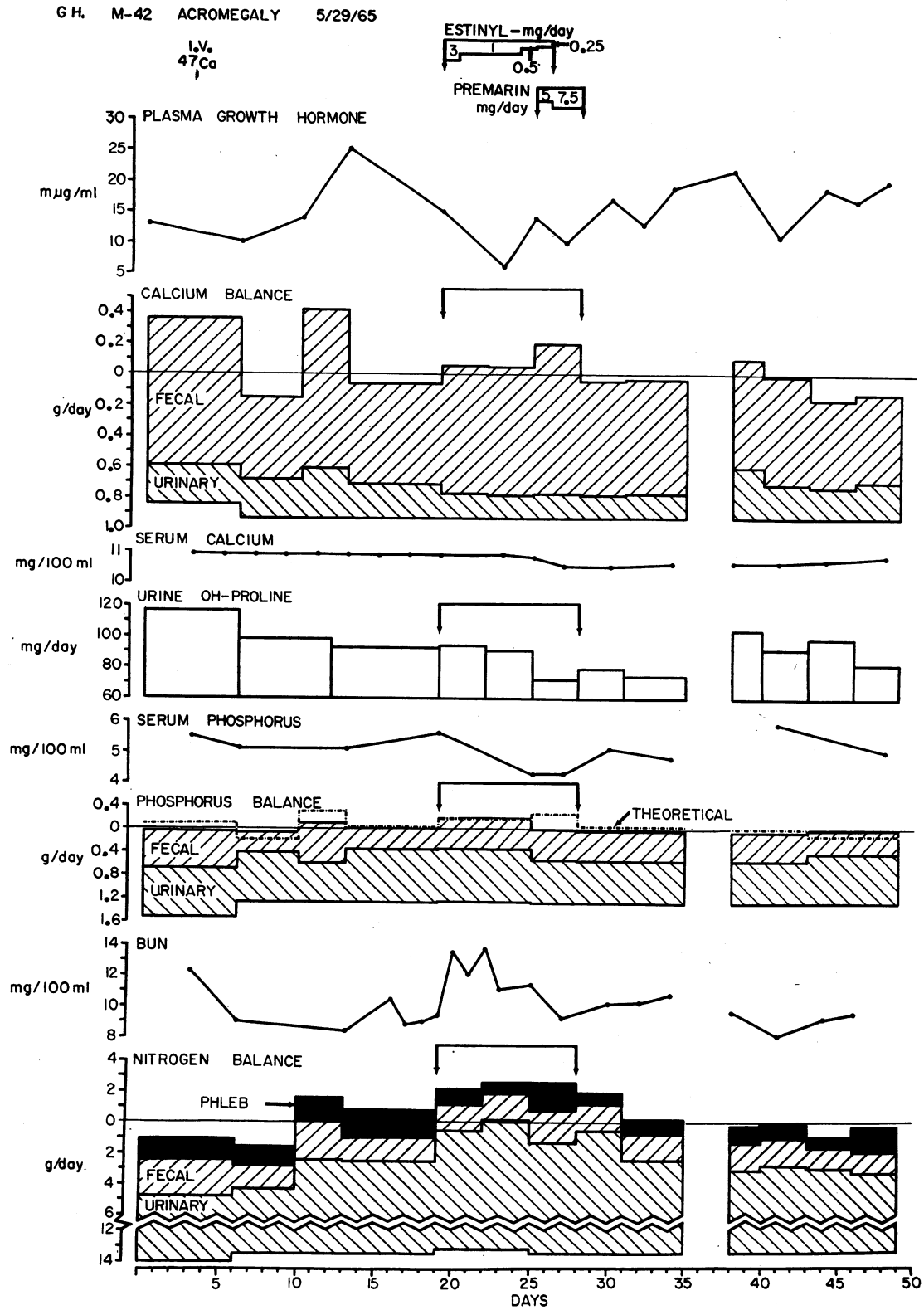

FIGURE 2 Metabolic data in patient G.H., a 42 yr old acromegalic male. Estinyl and (or) Premarin therapy reduced urinary calcium, urinary hydroxyproline, and serum phosphorus. There was a transient elevation of

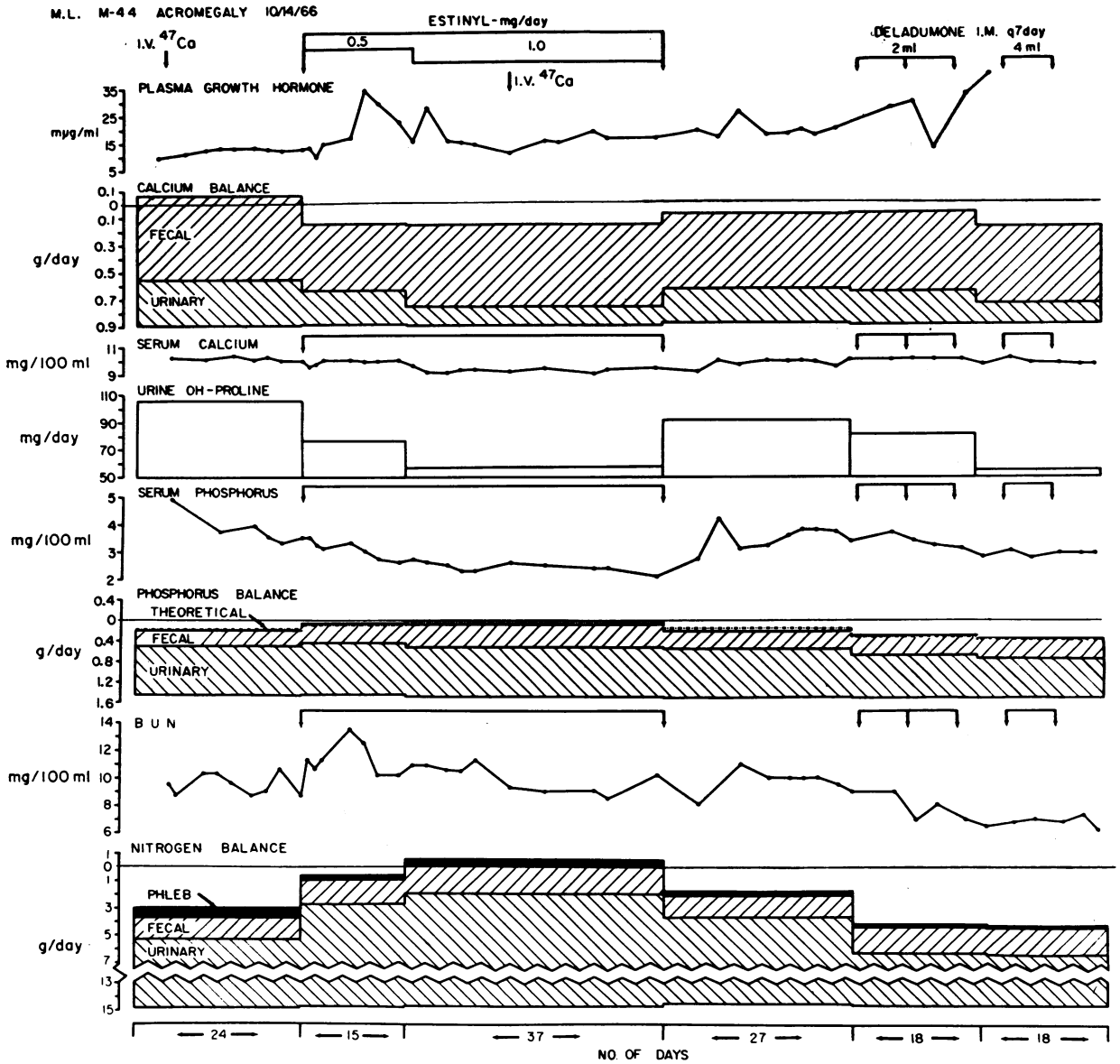

FIGURE 3 Metabolic data in patient M.L., a 44 yr old acromegalic male. Estinyl therapy lowered urinary calcium, urinary hydroxyproline, and serum phosphorus, while elevating urinary nitrogen, and transiently elevating blood urea nitrogen. Deladumone therapy produced similar effects upon urinary calcium, urinary hydroxyproline, and serum phosphorus, but a potent nitrogen-anabolic effect was observed which attributed to the testosterone-enanthate component of the drug. Elevated plasma growth hormone levels fluctuated independently of sex hormone administration.

unit (6, 7). In patient S.P., urinary and fecal calciums were determined by a modified ultramicro ethylenediaminetetraacetate titration method, with cal-red as indicator (8). In the other three patients, calcium determinations were performed by atomic absorption spectrophotometry, with the Perkin-Elmer model 303 instrument and a modification of the method of Willis (9). Blood sugars were determined by Technicon automated analysis with ferricyanide reduction. In patients S.P. and G.H., conventional stool-marking techniques were used at 6-day intervals, with carmine red capsules and laxation with bisacodyl or sennokot tablets. In patients M.L. and A.R., fecal marking was accomplished by

blood urea nitrogen, and a sustained elevation, during estrogen therapy, of urinary nitrogen, resulting in more negative nitrogen balance. Fluctuations in the elevated plasma growth hormone level were not grossly affected by estrogen therapy. The black areas labeled Phleb refer to the nitrogen loss in blood withdrawn for tests.

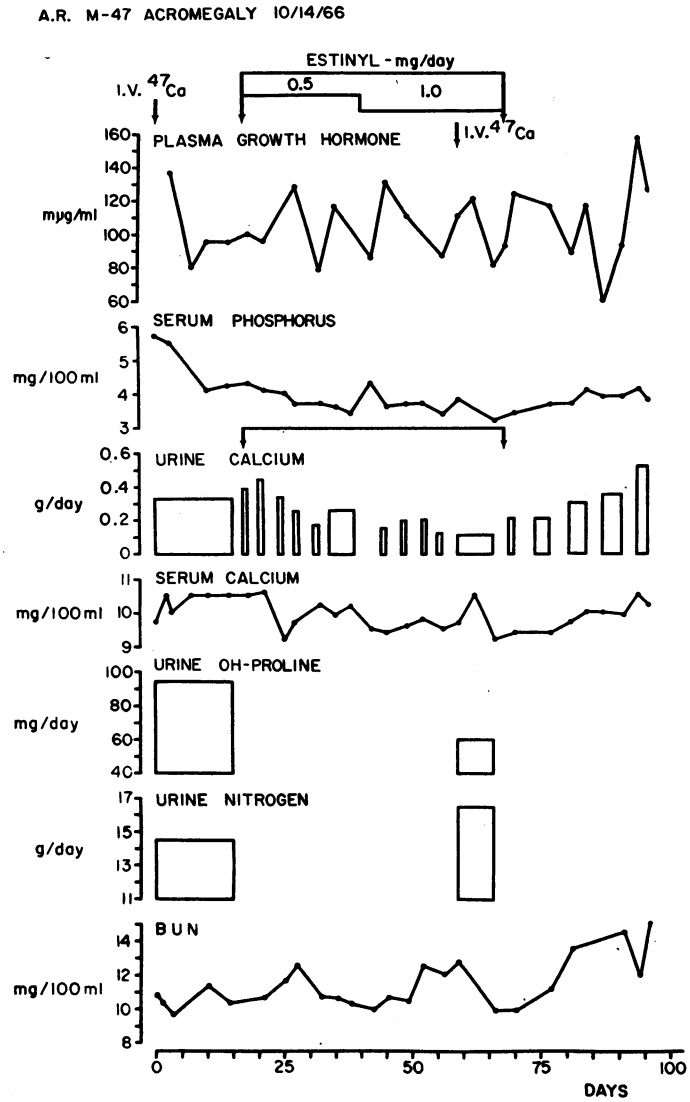

FIGURE 4 Metabolic data in patient A.R., a 47 yr old acromegalic male. Estinyl therapy lowered urinary calcium, urinary hydroxyproline, and serum phosphorus, while elevating urinary nitrogen. There were wide fluctuations of plasma growth hormone level, not influenced by estrogen administration. The complete metabolic balance data for calcium, phosphorus, and nitrogen during the two balance periods are not shown here but are listed in Table II.

daily oral administration of chromium sesquioxide. Fecal chromium recoveries were determined by atomic absorption spectrophotometry (10). The nitrogen balance data were corrected for losses caused by phlebotomy for chemistries and radiocalcium studies (6). Urinary hydroxyproline determinations were made by the method of Prockop and Udenfriend (11). Plasma growth hormone and insulin levels were determined by the radioimmunoassay method (12, 13). As described previously (7), radiocalcium kinetic parameters were derived from analysis of a simplified one-compartment model, averaging the results of two different methods of calculation based upon the same mathematical assumptions. It is now accepted that bone accretion rate as measured by this method is not a direct measure of bone formation rate, since it does not account for exchange processes involving multiple calcium compartments. Nonetheless, one-compartment estimations of bone accretion rate appear to be well correlated with microradiographic and tetracycline-labeled determinations of bone formation rate in metabolically different groups of animals (14) and are probably fairly reliable indices of changes in bone formation rate when the subject serves as its own control.

RESULTS

Plasma growth hormone data. In the four patients, it was evident that the mean values of plasma growth hormone were not significantly altered during estrogen therapy (Table I, Figs. 1–4). Estrogen therapy did not alter the failure of patients M.L. and A.R. to suppress plasma growth hormone levels after oral administration of glucose (Figs. 5 and 7). Both patients had a rise of growth hormone with insulin-induced hypoglycemia during estrogen therapy (Figs. 6 and 8). After discontinuance of estrogen, patient M.L. manifested unchanged growth hormone response to hypoglycemia (Fig. 6), while patient A.R. had a much lower and probably nonsignificant response (Fig. 8).

Throughout the studies, plasma growth hormone levels fluctuated by as much as ±50% of the mean values. These fluctuations may have been physiological in origin and perhaps partly due to lack of assay precision at high growth hormone levels. In one patient, G.H., whose mean growth hormone levels were comparable before and after cessation of estrogen therapy (15.4 mμg/ml and 14.8 mμg/ml, respectively), the first of three assay values obtained during 9 days of estrogen therapy was considerably lower (6 mμg/ml), but was not considered significant. In patient A.R., whose basal plasma growth hormone levels manifested especially extreme fluctuations, Estinyl therapy had no apparent effect. The mean assay values before (102 mμg/ml), during (103 mμg/ml), and after estrogen therapy (105 mμg/ml) were almost identical. In patient M.L., after discontinuance of Estinyl, and reestablishment of a postcontrol base line, weekly injections of 2 ml of Deladumone were begun (1 ml = testosterone enanthate, 90 mg + estradiol valerate, 4 mg), to ascertain if the androgen in a sexually neutral preparation would modify the action of estrogen. Allowing for the random fluctuations observed in this patient, we judged that the androgen-estrogen mixture also had no significant effect upon plasma growth hormone levels (Fig. 3).

Glucose tolerance, insulin secretion, and insulin sensitivity (Figs. 5–8). While the initial glucose tolerance test of patient M.L. was not clearly abnormal, there appeared to be definite improvement of glucose tolerance during estrogen therapy (Fig. 5). His postcontrol glucose tolerance test was definitely abnormal. Patient M.L.'s relatively normal insulin response to oral glucose was sharply increased during estrogen therapy and

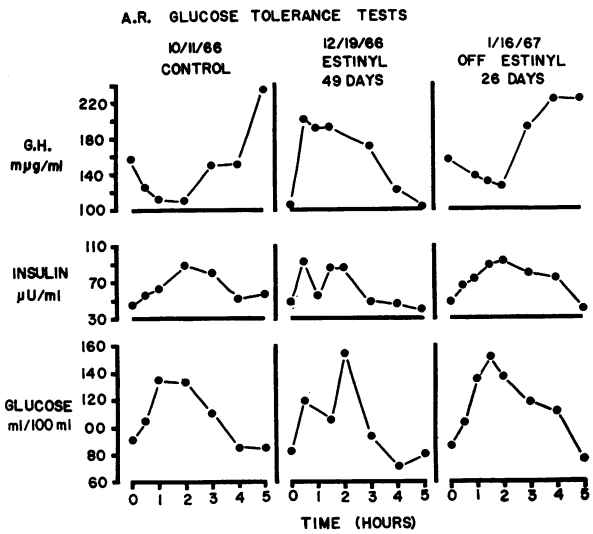

FIGURE 6 Effect of estrogen in patient A.R. upon oral glucose tolerance and upon plasma growth hormone and insulin response. While elevated plasma growth hormone levels were nonsuppressible throughout, there appeared to be late growth hormone elevations during the pre- and postcontrol tests and an early elevation during estrogen therapy. Glucose tolerance was moderately abnormal throughout, with elevated 2-hr blood sugars, but plasma insulin response appeared relatively normal and was unaffected by estrogen therapy.

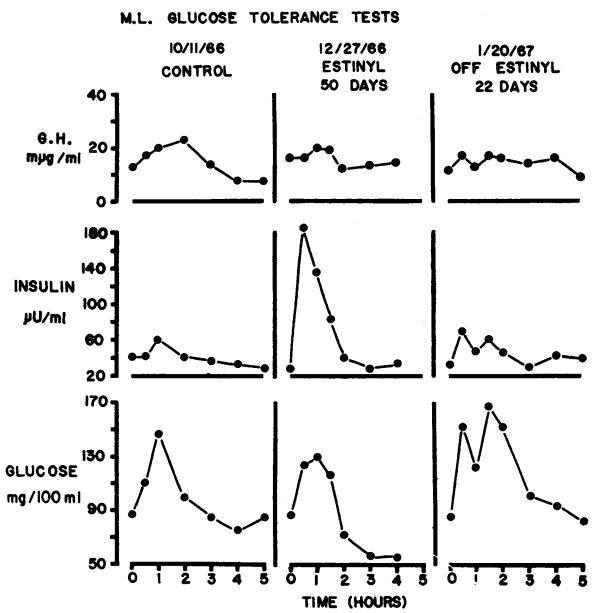

FIGURE 5 Effect of estrogen in patient M.L. upon oral glucose tolerance and upon plasma growth hormone and insulin response. Elevated plasma growth hormone levels were nonsuppressible throughout. Normalization of glucose tolerance is seen during estrogen administration, associated with marked elevation of insulin response.

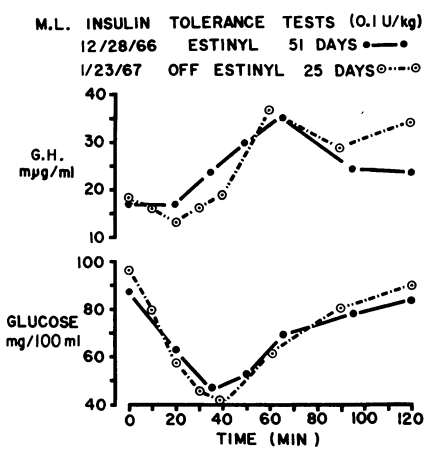

FIGURE 7 Effect of estrogen in patient M.L. upon insulin tolerance and hypoglycemic stimulation of plasma growth hormone. The patient was relatively normally sensitive to 0.1 U/kg regular insulin i.v., and estrogen had no apparent effect upon the reduction of blood glucose or elevation of plasma growth hormone.

TABLE II
Metabolic Balance Data and

Patient regimen		Calcium				Phos	
		Intake	Urine	Fecal	Balance	Intake	Urine
	(*days*)		(*g/day*)				(*g/*
S. P.							
Control	1–21	0.82	0.39	0.60	−0.17		
Estinyl, *1–3 mg/day*	22–194		0.21(99)*			On ward diet—approximate	
Estinyl, *3 mg/day*	195–214	0.85	0.14	0.75	−0.04		
G. H.							
Control	1–19	0.90	0.25	0.78	−0.13	1.35	0.85
Estinyl–Premarin‡	20–28	0.93	0.16	0.88	−0.11	1.26	0.86
Control	29–35	0.93	0.16	0.74	+0.03	1.27	0.72
Control	36–38					At home on	
Control	39–49	0.93	0.24	0.63	+0.06	1.27	0.79
M. L.							
Control	1–24	0.89	0.34	0.62	−0.07	1.45	0.95
Estinyl, *0.5 mg/day*	25–39	0.88	0.25	0.49	+0.14	1.44	1.00
Estinyl, *1.0 mg/day*	40–76	0.89	0.14	0.60	+0.15	1.48	0.96
Control	77–103	0.87	0.25	0.55	+0.07	1.49	0.95
Deladumone, 2 ml q *7 days*§	104–121	0.88	0.24	0.58	+0.06	1.50	0.83
Deladumone, 4 ml q *7 days*	122–139	0.88	0.15	0.56	+0.17	1.50	0.77
A. R.							
Control	1–15	0.96	0.33	0.48	+0.15	1.49	1.22
Control	16–17					At home on supervised diet	
Estinyl, *0.5 mg/day*	18–40		0.29(10)*				
Estinyl, *1.0 mg/day*	41–59		0.17(4)*				
Estinyl, *1.0 mg/day*	60–66	0.96	0.11	0.77	+0.08	1.49	1.21
Estinyl, *1.0 mg/day*	67–68					At home on supervised diet—	
Control	69–96		0.33(13)*				

* No. of urine determinations averaged.
‡ Estinyl, 3 mg, day 20; 1 mg, days 21–24; 0.5 mg, day 25; 0.25 mg, day 26. Premarin 5.0 mg, day 26; 7.5 mg, days 27–28.
§ Deladumone, 1 ml = testosterone enanthate 90 mg plus estradiol valerate, 4 mg.

returned to control levels after discontinuance of estrogen. Patient A.R. manifested moderately impaired glucose tolerance before, during, and after estrogen therapy, and his relatively normal insulin response remained essentially unchanged (Fig. 6). The fasting plasma insulin concentrations in both patients appeared to be slightly elevated (40 μU/ml in M.L.; 45 μU/ml in A.R.) and were not grossly altered by estrogen therapy. (The normal value for fasting insulin concentration in our laboratory is approximately 20 μU/ml.) Insulin sensitivity with respect to glucose, with regular insulin in a dosage of 0.1 U/kg, was not significantly altered by estrogen therapy in patient M.L. (Fig. 7) and may have increased in patient A.R. (Fig. 8). Neither patient was obviously insulin-resistant.

Calcium balance data (Tables I, II, and Figs. 1–4). Three of the four patients (S.P., M.L., and A.R.) were considered to be moderately hypercalciuric. In all four patients, estrogen therapy effected reductions of urinary calcium from initial levels of 0.39 g/day (S.P.), 0.25 g/day (G.H.), 0.34 g/day (M.L.), and 0.33 g/day (A.R.) to levels of 0.14 g/day, 0.16 g/day, 0.14 g/day, and 0.11 g/day, respectively. Two of the patients (S.P. and M.L.) had control calcium balances which were negative and which tended to become more positive during estrogen therapy. Patient A.R. manifested mildly positive calcium balances before and during estrogen therapy. In patient G.H., wide oscillations of fecal calcium values prevented assessment of changes in calcium balance.

Radiocalcium kinetic data (Table I). Compared with an average "normal" accretion rate of 0.44 g/day calcu-

Urinary Hydroxyproline Excretion

phorus			Nitrogen					Urinary hydroxy-
Fecal	Balance	Theor-Bal.	Intake	Urine	Fecal	Phlebotomy	Balance	proline
day)					*(g/day)*			*(mg/day)*
calcium intake, *800 mg/day*								
0.50	0	−0.04	13.7	10.0	2.0	1.5	+0.2	102
0.53	−0.13	−0.22	13.3	12.7	1.8	1.2	−2.4	86
0.52	+0.03	−0.05	13.5	11.9	1.7	0.8	−0.9	77
supervised diet								
0.45	+0.03	+0.05	13.5	10.4	1.6	1.1	+0.4	93
0.41	+0.19	+0.17	14.8	9.5	1.6	0.7	+3.0	106
0.38	+0.06	+0.10	14.7	12.0	1.8	0.3	+0.6	77
0.44	+0.08	+0.03	14.6	12.7	2.0	0.5	−0.6	57
0.34	+0.20	+0.15	14.4	10.8	1.6	0.3	+1.7	92
0.38	+0.29	+0.31	14.5	8.3	1.9	0.2	+4.1	82
0.38	+0.35	+0.36	14.5	8.2	1.9	0.2	+4.2	55
0.37	−0.10	−0.02	15.7	14.5	1.6	0.9	−1.3	94
0.83	−0.55	−0.26	15.7	16.5	2.9	0.7	−3.7	60
approximate calcium intake, *1.0 g/day*								

lated by us previously from composite normal data (7), the control accretion rates were elevated in the three patients studied (S.P., M.L., and A.R.). In each patient, the marked reductions of urinary calcium effected by estrogen therapy were associated with reductions of accretion rate, of exchangeable calcium pool, and of total radioisotope excretion. In two patients (S.P. and A.R.), intestinal calcium absorption was sharply reduced. The accretion rates declined from 0.85 g/day to 0.52 g/day (S.P.), from 1.41 g/day to 1.03 g/day (M.L.), and from 1.69 g/day to 1.28 g/day (A.R.). The rapidly exchangeable calcium (2 hr pool) values showed no consistent pattern of alteration.

Hydroxyproline excretion (Table II, Figs. 1–4). In patients G.H., M.L., and A.R., average control 24-hr urinary hydroxyproline values of 102, 106, and 94 mg, respectively, were reduced by estrogen therapy to minimum levels of 73 mg/day, 57 mg/day, and 60 mg/day. In patient M.L., whose urinary hydroxyproline fell to 57 mg/day during the last 37 of 52 days of estrogen therapy, the discontinuance of estrogen was followed by a rise of hydroxyproline excretion to 92 mg/day averaged over a 27 day postcontrol period. The institution in patient M.L. of weekly intramuscular estrogen-androgen therapy as Deladumone was again followed by a reduction of urinary hydroxyproline to 55 mg/day, averaged over the last 18 days of an observation period of 36 days.

Serum phosphorus and the nitrogen balance (Table II, Figs. 1–4). In three of the four patients, (G.H., M.L., and A.R.), estrogen therapy reduced serum phosphorus concentration from levels of 5 mg/100 ml or higher to 4 mg/100 ml or lower. In the fourth patient

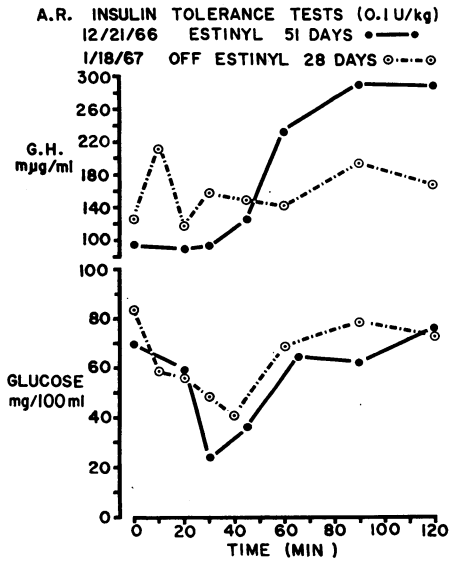

FIGURE 8 Effect of estrogen in patient A.R. upon insulin tolerance and hypoglycemic stimulation of plasma growth hormone. Estrogen therapy appeared to increase both the insulin sensitivity with respect to blood glucose and the hypoglycemia-stimulated rise of plasma growth hormone.

(S.P.), serum phosphorus only occasionally fell below 4 mg/100 ml during estrogen therapy. In two patients (G.H. and M.L.), estrogen therapy resulted in initial transient elevations of the blood urea nitrogen and negatively influenced the nitrogen balance. Patient M.L., who was in strongly positive nitrogen balance before and after estrogen therapy, manifested slightly negative nitrogen balance during estrogen therapy. In patient A.R., during the second brief balance period at the end of estrogen therapy, there occurred increased urinary nitrogen balance compared with the control period. In patient S.P., the effect of estrogen upon nitrogen balance was obscured by erratic fluctuations in nitrogen intake during the second study, which invalidated the nitrogen data.

DISCUSSION

The effectiveness of estrogen therapy in reducing hypercalciuria and hydroxyprolinuria in our acromegalic patients may reflect the known capacity of estrogen to inhibit bone resorption (15). Hypercalciuria and negative calcium balances have been attributed to excessive bone resorption in acromegaly (16). Likewise, the elevation of urinary hydroxyproline excretion seen in acromegalic patients (17), growing children (17), and in normal adults given growth hormone (18) suggests increased bone resorption (and [or] increased collagen turnover). Increased resorptive activity would be expected during the bone overgrowth caused by excess of growth hormone. Sequential studies of normal bone growth have clearly shown that activation of bone resorption is necessarily closely coupled to bone formation (19). Overall, increased bone formation in acromegaly, as evidenced by elevated radiocalcium accretion rates in these studies and in others (20), probably predominates over increased bone resorption, since the acromegalic skeleton is larger and heavier than normal. The reduction by estrogen of radiocalcium bone accretion rates in our patients may partially reflect a coupled response to a primary inhibition of elevated bone resorption (7). However, it is probably also due to antagonism of the bone-formation–stimulating property of growth hormone. Estrogen therapy reduces elevated plasma sulfation factor in acromegaly (21), and partially inhibits growth hormone stimulation of epiphyseal growth in the hypophysectomized rat (22). Androgens may share this property, as shown by the reduction of bioassayable epiphyseal-stimulating activity in the blood of a testosterone-treated acromegalic patient (23).

The suppression by estrogen therapy of elevated serum phosphorus levels in acromegalic patients has been repeatedly observed in the past (1, 2, 21). Serum phosphorus levels are high in growing children (24) and are readily elevated by administration of growth hormone to normal patients (18, 25), an effect attributed to increased tubular reabsorption of phosphate (25). Elevation by estrogen therapy of the blood urea nitrogen levels and negative effects upon nitrogen balance in acromegaly have not been previously described. Estrogen was reported not to affect nitrogen balance in two acromegalic patients (26), but the patients were in strongly positive nitrogen balance on respective intakes of 17.5 and 21 g of nitrogen per day. Our patients were consuming much lower nitrogen intakes of 13.5 and 14.5 g/day, respectively.

The variable patterns of glucose tolerance, insulin secretion, and insulin sensitivity observed in our patients, and by others in acromegaly, obscure the interpretation of estrogenic effects. As observed in patient M.L., estrogen therapy clearly ameliorated abnormal glucose tolerance in each of five female acromegalic patients described by McCullagh, Beck and Schaffenburg (3), and initially seemed to improve the glucose tolerance of the four patients described by Mintz, Finster, and Josimovich (4). In the latter study, three of the four patients initially manifested hyperinsulinemic responses to oral glucose, and all three patients had reduction of insulin response during estrogen therapy. In contrast, our two patients did not manifest high insulin responses to glucose in the control studies, but patient M.L. manifested a hyperinsulinemic response after estrogen therapy. Hyperinsulinemia after oral glucose occurs only in about 50% of acromegalic patients and is not related to the normality or abnormality of glucose tolerance tests (27, 28). The finding of increased insulin

response in patient M.L. after estrogen therapy resembles the augmentation of insulin response to intravenous glucose reported with estrogen-progestin contraceptive drug therapy (29). The mechanism of this effect is unclear, but it is associated with deterioration of glucose tolerance, which was not seen in patient M.L. Only one of our two patients (A.R.) appeared somewhat more sensitive to insulin while on estrogen threapy, a finding described in acromegalic males treated with estrogen (2), and in two of the four females treated by Mintz et al. (4).

As observed in each of our four patients, in four of five acromegalic patients reported in the literature (4, 5), estrogen therapy did not reduce elevated basal levels of radioimmunoassayable growth hormone. One patient (4) had an apparent decrease in plasma growth hormone from an initial level of approximately 50 mμg/ml to 15 mμg/ml after 1 month of estrogen therapy. These values were apparently only single determinations and may not significantly differ from expected random fluctuations. The failure of estrogen therapy in patients M.L. and A.R. to reduce growth hormone levels in the basal state, after oral glucose, or after insulin-induced hypoglycemia, suggests that the alterations of urinary calcium and hydroxyproline excretion, serum phosphorus, blood urea nitrogen, nitrogen balance, and of radiocalcium kinetic parameters were not due to over-all reductions in growth hormone secretion, but rather to estrogenic effects at the periphery. This conclusion is consistent with the results of other metabolic studies in our unit ([18] and to be reported) which demonstrate estrogenic antagonism of the effects of growth hormone administered to nonacromegalic patients.

ACKNOWLEDGMENTS

We wish to express our appreciation to Dr. Seymour M. Glick for performing the growth hormone radioimmunoassay determinations in patients S.P. and G.H., and to Dr. Solomon A. Berson and to Dr. Glick for providing us with the laboratory facilities for determinations in patients M.L. and A.R. We are indebted to Doctors Grace Bellotti, Sheldon Simon, and Eckehardt Wiedemann for help in the care of patients. We are grateful for the unstinting work of the Metabolic Unit staff: Miss Rose Saffo and Mrs. Sarah Gibbs, nurses; Mrs. Harriet Kahn and Miss Kathryn Nethercott, dieticians; Mr. John A Saeli and Miss Bernice Middleton, biochemists; Mrs. Evelyn Edwards and Mrs. Cleo Bernard, dietetic aides; Mrs. Maureen Smyth and Mrs. Mercedes Torres, secretaries.

REFERENCES

1. Kirklin, O. L., and R. M. Wilder, 1936. Follicular hormone administration in acromegaly. *Proc. Mayo Clin.* **11**: 121.
2. Albright, F., E. C. Reifenstein, Jr., A. P. Forbes, and F. C. Bartter. 1946. Effect of estrogen in acromegaly. *In* Conference on Metabolic aspects of Convalescence: Transactions of the Fourteenth Meeting. Josiah Macy, Jr. Foundation, New York. 102.
3. McCullagh, P., J. C. Beck, and C. A. Shaffenburg. 1955. Control of diabetes and other features of acromegaly following treatment with estrogens. *Diabetes.* **4**: 13.
4. Mintz, D. H., J. L. Finster, and J. B. Josimovich. 1967. Effect of estrogen therapy on carbohydrate metabolism in acromegaly. *J. Clin. Endocrinol. Metab.* **27**: 1321.
5. Deller, J. J., Jr., V. C. DiRaimondo, G. M. Grodsky, and P. H. Forsham. 1966. Acromegaly. The effects of various steroid hormones on the insulin-induced growth hormone response. *Calif. Med.* **104**: 1.
6. Schwartz, E., W. V. Chokas, and V. A. Panariello. 1964. Metabolic balance studies of high calcium intake in osteoporosis. *Amer. J. Med.* **36**: 233.
7. Schwartz, E., V. A. Panariello, and J. Saeli. 1965. Radioactive calcium kinetics during high calcium intake in osteoporosis. *J. Clin. Invest.* **44**: 1547.
8. Bachra, B., A. Dauer, and A. E. Sobel. 1958. The complexometric titration of micro- and ultramicroquantities of calcium in blood, serum, urine and inorganic salt solutions. *J. Clin. Chem.* **4**: 107.
9. Willis, J. B. 1961. Determination of calcium and magnesium in urine by atomic absorption spectroscopy. *Anal. Chem.* **33**: 556.
10. Williams, C. H., David, D. J., and O. Iismaa. 1962. The determination of chronic oxide in feces samples by atomic absorption spectrophotometry. *J. Agr. Sci.* **59**: 381.
11. Prockop, D. J., and S. Udenfriend. 1960. A specific method for the analysis of hydroxyproline in tissues and urine. *Anal. Biochem.* **1**: 228.
12. Yalow, R. S., and S. A. Berson. 1960. Immunoassay of endogenous plasma insulin in man. *J. Clin. Invest.* **39**: 1157.
13. Glick, S. M., J. Roth, R. S. Yalow, and S. A. Berson. 1963. Immunoassay of human growth hormone in plasma. *Nature (London).* **199**: 784.
14. Riggs, B. L., Jowsey, J., Ackerman. E., and J. B. Hazelrig. 1967. Ability of ^{47}Ca kinetic analysis to discriminate metabolic states affecting bone formation in dogs. *Metabolism.* **16**: 1064.
15. Gardner, W. V., and C. A. Pfeiffer. 1943. Influence of estrogens and androgens on the skeletal system. *Physiol. Rev.* **23**: 139.
16. Albright, F., and E. C. Reifenstein, Jr. 1948. The Parathyroid Glands and Metabolic Bone Disease. The Williams & Wilkins Company, Baltimore. 188.
17. Jasin, H. E., C. W. Fink, W. Wise, and M. Ziff. 1962. Relationship between urinary hydroxyproline and growth. *J. Clin. Invest.* **41**: 1928.
18. Schwartz, E., E. Echemendia, and M. Schiffer. 1967. Peripheral metabolic antagonism of estrogen and human growth hormone. *J. Clin. Invest.* **46**: 1115. (Abstr.)
19. Sissons, H. A. 1953. Experimental determination of rate of longitudinal bone growth. *J. Anat.* **87**: 228.
20. Bell, N. H., and F. C. Bartter. 1967. Studies of 47-Ca metabolism in acromegaly. *J. Clin. Endocrinol. Metab.* **27**: 178.
21. Almqvist, S., D. Ikkos, and R. Luft. 1961. Studies on Sulfation factor (SF) activity of human serum. The effects of estrogen and x-ray therapy on serum SF activity in acromegaly. *Acta Endocrinol.* **37**: 138.
22. Josimovich, J. B., D. H. Mintz, and J. L. Finster. 1967. Estrogenic inhibition of growth hormone-induced tibial epiphyseal growth in hypophysectomized rats. *Endocrinology.* **81**: 1428.
23. Kinsell, L. W., G. D. Michaels, C. H. Li, and W. E. Larsen. 1948. Studies in growth. I. Interrelationship between pituitary growth factor and growth-promoting

androgens in acromegaly and gigantism. II. Quantitative evaluation of bone and soft tissue growth in acromegaly and gigantism. *J. Clin. Endocrinol. Metab.* **8**: 1013.
24. Albright, F., and E. C. Reifenstein, Jr. 1948. The Parathyroid Glands and Metabolic Bone Disease. The Williams & Wilkins Company, Baltimore. 4.
25. Corvilain, J., and M. Abramow. 1962. Some effects of human growth hormone on renal hemodynamics and on tubular phosphate transport in man. *J. Clin. Invest.* **41**: 1230.
26. Albright, F., and E. C. Reifenstein, Jr. 1948. The Parathyroid Glands and Metabolic Bone Disease. The Williams & Wilkins Company, Baltimore. 193.
27. Beck, P., D. S. Schalch, M. L. Parker, D. M. Kipnis, and W. H. Daughaday. 1965. Correlative studies of growth hormone and insulin plasma concentrations with metabolic abnormalities in acromegaly. *J. Lab. Clin. Med.* **66**: 766.
28. Karam, J. H., G. M. Grodsky, F. C. Pavlatos, and P. H. Forsham. 1965. Critical factors in excessive serum-insulin response to glucose. Obesity in maturity-onset diabetes and growth hormone in acromegaly. *Lancet.* **1**: 286.
29. Spellacy, W. N., K. L. Carlson, and S. A. Birk. 1967. Carbohydrate metabolic studies after six cycles of combined type oral contraceptive tablets. *Diabetes.* **16**: 590.