JCI The Journal of Clinical Investigation

Studies in Cushing's Syndrome. II. Adrenal Weight-Maintaining Activity in the Plasma of Patients with Cushing's Syndrome

Joseph W. Jailer, … , Donald Longson, Nicholas P. Christy

J Clin Invest. 1957;36(11):1608-1614. https://doi.org/10.1172/JCI103560.

Research Article

STUDIES IN CUSHING'S SYNDROME. II. ADRENAL WEIGHT-MAINTAINING ACTIVITY IN THE PLASMA OF PATIENTS WITH CUSHING'S SYNDROME [1]

By JOSEPH W. JAILER, DONALD LONGSON,[2] AND NICHOLAS P. CHRISTY [3]

(From the Departments of Medicine, and Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, N. Y.)

(Submitted for publication June 28, 1957; accepted July 25, 1957)

Patients with Cushing's syndrome due to bilateral adrenal cortical hyperplasia have been reported by several investigators to show an exaggerated plasma 17-hydroxycorticosteroid response to administered ACTH (1–3). Although pituitary corticotropin has been suspected of playing an etiologic role in this disorder, previous workers have generally failed to detect elevated levels of ACTH in the blood of patients with Cushing's syndrome (4–7). Furthermore, the prolonged administration of ACTH to normal subjects has not been observed to cause adrenal cortical hyper-response comparable to that found in bilateral adrenal hyperplasia (8, 9).

In search of an explanation for this adrenal hyper-responsiveness, the possibility has been investigated of another type of corticotropic activity, different from the usual ACTH (10–12). The purpose of this communication is to present data which indicate the occurrence of adrenal weight-maintaining activity in the plasma of patients with Cushing's syndrome associated with adrenal hyperplasia.

METHODS AND MATERIALS

Plasma for assay was obtained from several groups of patients (*vide infra*) in the following manner. Samples of 100 to 200 ml. of blood were collected in iced tubes with heparin added, plasma was quickly separated in a cold centrifuge, and then frozen and stored at −20° C. in small tubes containing sufficient material (6 to 9 ml.) for a single day's injection. In three instances, plasma was lyophilized and reconstituted with distilled water to the original volume.

The assay method used was a modification of that devised by Simpson, Evans, and Li in which corticotropic activity was measured by the ability of a substance to prevent adrenal atrophy in the rat following hypophysectomy (13). Male Sprague-Dawley rats were hypophysectomized at 30 days of age. Injections of the plasma to be assayed were begun 24 hours later. The dosage of plasma was 0.3 ml. given subcutaneously three times a day for nine days, making a total dose of 8 to 9 ml. per rat. Plasma from each individual patient (or in some instances, from each plasma pool, *vide infra*) was administered to a group of six to nine rats. The control for each group of experimental rats was a group of six to ten animals of exactly the same age, hypophysectomized on the same day, and maintained identically throughout each experimental period, except that no injections were given. As an additional control for patients' plasma, groups of hypophysectomized rats were given plasma from normal human subjects (*vide* Results, Section 3). On the tenth day, animals were sacrificed. The adrenals were removed, dissected free of fat, wet-weighed, fixed in formalin, stained with Sudan Black, and sectioned for histologic study. Completeness of hypophysectomy was estimated by testicular size, and in six instances, by gross examination of the sella. All animals judged to be incompletely hypophysectomized were discarded.

RESULTS

1. *Bilateral adrenal hyperplasia*

Plasma for assay was obtained from nine patients with this disease. All showed the typical clinical and laboratory features of Cushing's syndrome. In six patients, the diagnosis of adrenal hyperplasia was proved at operation, and in the remaining three (N.Co., R.P., B.S.) no adrenal tumor was detected by presacral aerography. Eight of the nine patients (with the exception of S.A.) showed the typical plasma 17-hydroxycorticosteroid hyper-response to a standardized ACTH test (2, 9).

Table I shows results of the assay for adrenal weight-maintaining activity in the plasma of these nine patients. Eight of the nine plasma specimens (with the exception of patient B.S.) were found to prevent adrenal atrophy to a significant degree.

[1] Aided by Grants A-195(C) and A-1384 from the National Institute of Arthritis and Metabolic Diseases.

[2] Dickenson Traveling Fellow, University of Manchester, England, and Schering Fellow in Endocrinology.

[3] John and Mary R. Markle Scholar in Medical Science.

TABLE I

Effect of plasma injection upon adrenal weight of hypophysectomized rats: Patients with Cushing's syndrome due to bilateral adrenal hyperplasia

Patient	Injected animals			Non-injected animals			Difference in paired adrenal weight	"t"*
	No. animals	Mean paired adrenal weight	S.D.	No. animals	Mean paired adrenal weight	S.D.		
		mg.			mg.		mg.	
F. S.	5	11.9	1.47	9	7.3	0.74	4.6	8.0
S. L.	6	10.7	1.27	9	7.3	0.74	3.4	6.4
N. Co.	6	9.8	1.34	6	7.6	0.84	2.2	3.4
C. P.	7	9.9	1.29	9	7.6	0.55	2.3	4.8
B. W.†	4	9.1	1.36	3	6.5	0.47	2.6	3.0
H. H.†	3	11.3	2.82	6	7.7	1.04	3.6	3.0
R. P.	8	11.0	1.09	9	8.0	0.41	3.0	7.6
S. A.	7	7.9	1.11	10	6.5	0.99	1.4	2.8
B. S.	6	8.9	1.73	6	7.8	0.30	1.1	1.5
Average		10.1			7.4		2.7	7.40‡

* Value of "t" here and in subsequent tables was computed by assuming equal variability in the injected and non-injected groups of animals (29).

† The small number of animals was the result of deaths during the injection period.

‡ The differences in mean paired adrenal weights yield a S.D. of 1.10 and consequently the S.E. of the average difference is 0.37 so that "t" is 7.40, significant at the 1 per cent level. If the differences in numbers of animals and the differences in variability in the injected versus the non-injected groups are taken into account by applying a weighting procedure, the value of "t" is nearly identical (7.2).

It should be pointed out that patients C.P. and H.H. had undergone bilateral subtotal adrenalectomy one and one-half and three years before the plasma was obtained for assay.

Since the numbers of animals were necessarily small (being limited by the amount of plasma available), data from individual experiments were pooled for additional statistical analysis. The average difference in adrenal weight between injected and non-injected animals was 2.7 mg. with a "t" value of 7.4 (Table I). The difference was significant at the 1 per cent level. It was of interest that injected animals in this and subsequent groups generally showed greater final body weights than uninjected animals, the difference averaging about 6 Gm. (In this group, average weight gain of injected animals was 9 Gm.; of uninjected controls, 4 Gm.) This was true regardless of whether the plasma contained any activity in maintaining adrenal weight or not. Calculation of the average correlation coefficient between body weight and adrenal weight gave an "r" value of 0.289, which is not significant (average slope, 0.04). The greater final body weight of injected animals could thus account for only about 0.2 mg. of the 2.7 mg. difference in adrenal weights.

It is recognized that the degree of adrenal weight maintenance is modest, with an average adrenal weight of 10.1 mg. in injected animals as compared with 7.4 mg. in the controls. However, when similarly hypophysectomized animals were injected with a standard ACTH preparation in massive doses, *i.e.*, 1 I.U. daily in three divided doses for nine days, adrenal weight averaged only 14.9 mg. Further, corticotropic material has been estimated to be present in amounts of the order of milliunits or fractions of milliunits per 100 ml. of human plasma (5, 14, 15), and each experimental animal in the present study received but 8 to 9 ml. plasma.

Histologic study of adrenal glands from animals injected with plasma revealed persistence of the sudanophobic zone, which was abolished by ACTH administration.

Studies performed in two patients (F.S. and H.H.) employing three animals in each assay failed to reveal adrenal ascorbic acid-depleting activity in plasma when tested by the Munson modification (16) of the Sayers technique (17).

2. *Adrenal cortical tumor*

The data obtained from the assay of plasma from three patients with documented adrenal cortical adenoma or carcinoma are shown in Table II. None of the three had previously shown excessive responses of plasma corticosteroid levels to the

TABLE II

Effect of plasma injection upon adrenal weight of hypophysectomized rats: Patients with Cushing's syndrome due to adrenal cortical tumor

Patient	Injected animals			Non-injected animals			Difference in paired adrenal weight	"t"*
	No. animals	Mean paired adrenal weight	S.D.	No. animals	Mean paired adrenal weight	S.D.		
		mg.			*mg.*		*mg.*	
V. C. (adenoma)	5	8.7†	1.08	10	9.1†	0.99	0.4	0.3
C. C. (carcinoma)	7	8.1	0.46	10	7.6	0.87	0.5	1.6
A. G. (carcinoma)	5	9.3	0.56	9	8.4	1.11	0.9	2.0

* P in each case > 0.05.

† The greater adrenal weights found in the study of plasma from patient V. C. may be related to the fact that this experiment was terminated after seven days of injection instead of nine days because of a large number of animal deaths. Post-hypophysectomy atrophy of the adrenal would not be expected to have reached its plateau within a seven day period.

standardized ACTH test (9), and no adrenal weight-maintaining activity could be demonstrated in their plasma.

3. *Normal subjects*

Table III shows results of the assay of plasma from normal individuals. The first specimen was taken from a single normal subject, while the remaining three specimens were pools of approximately equal plasma samples taken from three to five normal individuals. No significant increase of adrenal weight could be shown in the injected animals. Grouped data revealed an average difference in adrenal weight of injected versus uninjected animals of 0.05 mg. with a "t" value of 0.19 (not significant).

4. *Primary adrenal cortical insufficiency*

A summary of the results of assay of plasma from patients with adrenal insufficiency, either spontaneous or following bilateral total adrenalectomy, is shown in Table IV. Most assay methods have shown elevated levels of corticotropin in the blood of such patients (4–6, 18). It was therefore of interest to learn if plasma from adrenal-insufficient subjects also contained adrenal weight-maintaining activity. Blood was obtained and plasma pooled from five such patients whose exogenous steroid (cortisone) had been discontinued six to eight hours before blood was taken. Table IV shows that no adrenal weight-maintaining activity was detectable. This experiment does not offer conclusive evidence for the absence of adrenal

TABLE III

Effect of plasma injection upon adrenal weight of hypophysectomized rats: Normal subjects

Subject	Injected animals			Non-injected animals			Difference in paired adrenal weight	"t"
	No. animals	Mean paired adrenal weight	S.D.	No. animals	Mean paired adrenal weight	S.D.		
		mg.			*mg.*		*mg.*	
N. C.	6	8.0	0.64	6	7.6	0.84	0.4	0.9
Pool No. 1	9	7.8	1.12	5	7.6	0.32	0.2	0.4
Pool No. 2	7	9.4	1.19	10	9.1	0.98	0.3	0.6
Pool No. 3	6	7.7	0.99	9	8.4	1.11	0.7	1.2

Average difference in mean paired adrenal weights, injected versus control	0.05 mg.
S.E. of the difference (based on S.D.'s of individual experiments)	0.27
"t"	0.19
Degrees of freedom	50
p	>0.7

TABLE IV

Effect of plasma injection upon adrenal weight of hypophysectomized animals: Patients with adrenocortical insufficiency

	Injected animals			Non-injected animals				
	No. animals	Mean paired adrenal weight	S.D.	No. animals	Mean paired adrenal weight	S.D.	Difference in paired adrenal weight	"t"*
		mg.			*mg.*		*mg.*	
Plasma pool	6	8.1	0.52	5	7.6	0.33	0.5	1.8

* $p > 0.4$.

weight-maintaining activity from Addisonian blood since, as Bethune, Ganong, Hume, and Nelson have shown, the time elapsed after steroid withdrawal may not have been adequate to permit "rebound" of anterior pituitary function from the suppressive effect of the steroid (18). However, studies with an aliquot of this pooled plasma revealed a decrease of 43 per cent in the adrenal ascorbic acid of two animals as compared with two controls (16).

5. *Pregnancy*

Since women in the third trimester of normal pregnancy had been previously shown to exhibit exaggerated plasma 17-hydroxycorticosteroid response to ACTH [4] like that found in Cushing's syndrome (2), a study of adrenal weight-maintaining activity in the plasma of this group was also made. Table V shows results of assay of plasma pooled from three groups of pregnant women, each group comprising two to three women. All three pools showed significant activity.

6. *Acromegaly*

Because several workers (10–12) had observed adrenal weight-maintaining properties in pituitary growth hormone fractions, studies of plasma were

[4] There is evidence that this "excessive response" may be in part related to a delayed disposal of hydrocortisone from plasma of pregnant women (19, 20).

TABLE V

Effect of plasma injection upon adrenal weight of hypophysectomized animals: Women in the third trimester of normal pregnancy

	Injected animals			Non-injected animals				
	No. animals	Mean paired adrenal weight	S.D.	No. animals	Mean paired adrenal weight	S.D.	Difference in paired adrenal weight	"t"*
		mg.			*mg.*		*mg.*	
Pool No. 1	5	10.4	1.77	10	7.6	0.87	2.8	3.0
Pool No. 2	8	9.7	1.16	10	6.5	0.99	3.2	5.8
Pool No. 3	6	11.0	1.67	6	7.8	0.28	3.2	4.2

* p in each case < 0.01.

TABLE VI

Effect of plasma injection upon adrenal weight of hypophysectomized rats: Two patients with active acromegaly

	Injected animals			Non-injected animals				
Patient	No. animals	Mean paired adrenal weight	S.D.	No. animals	Mean paired adrenal weight	S.D.	Difference in paired adrenal weight	"t"*
		mg.			*mg.*		*mg.*	
F. N.	6	9.9	1.13	9	7.6	0.55	2.3	4.7
G. E.	8	10.9	0.89	9	8.0	0.41	2.9	8.3

* p in both instances < 0.01.

TABLE VII

Effect of growth hormone upon adrenal weight of hypophysectomized rats

Injected animals			Non-injected animals				
No. animals	Mean paired adrenal weight	S.D.	No. animals	Mean paired adrenal weight	S.D.	Difference in paired adrenal weight	"t"*
	mg.			*mg.*		*mg.*	
5	10.8	0.53	7	8.8	1.09	2.0	4.2

* $p < 0.01$.

also made in two patients with "active" acromegaly. Table VI shows significant adrenal weight-maintaining activity in the plasma of both patients. It should be pointed out that neither of these two subjects had exaggerated plasma corticosteroid responses to ACTH.

7. *Growth hormone*

Investigation of the effect of bovine growth hormone (supplied by the National Institutes of Health) was carried out. Three mg. of this preparation were administered daily to a group of hypophysectomized rats, the adrenal weight assay being carried out as described above. The data presented in Table VII indicate that this growth hormone preparation contained significant adrenal weight-maintaining activity ($p < 0.01$) as previously demonstrated (10–12). The sudanophobic zone of the adrenal cortex persisted. One mg. of this somatotropin contained no corticotropic activity, as determined by the method of Saffran and Schally (21).

DISCUSSION

The data presented indicate that plasma from patients with Cushing's syndrome due to bilateral adrenal hyperplasia exhibited adrenal weight-maintaining activity as demonstrated by assay in hypophysectomized rats. Plasma of pregnant women in the third trimester of pregnancy and of two patients with active acromegaly showed similar activity. It is not possible at this time to ascribe this weight-maintaining effect to a hormone with any certainty, since it is recognized that the demonstration of a substance of possibly hormonal nature conventionally rests upon the finding of its differential potency at increasing dose levels. Attempts to assay patients' plasma at doses greater than 0.9 ml. per rat per day in this study proved impractical since the larger amounts resulted in the death of nearly all the test animals. Construction of a dose-response curve must await purification of plasma.

A number of substances might be implicated to explain the partial maintenance of adrenal weight observed in these studies: 1) steroids of the adrenal cortex, 2) corticotropin, and 3) growth hormone. Certain steroids have been shown to cause increases in adrenal weight (22, 23), but in the present study it seems unlikely that elevated plasma adrenal steroid levels were responsible for the observed increase in adrenal weight. In the first place, no adrenal weight increase was induced by the plasma from patients with adrenal cortical tumors whose resting plasma corticosteroid levels were as high as or higher than those of patients with adrenal hyperplasia.[5] Second, adrenal weight-maintaining activity was found in plasma of two patients who had undergone bilateral adrenalectomy before the assay was carried out. Third, cortisone (a prototype of the class of steroid elevated to the greatest degree in Cushing's syndrome) has been shown to have no weight-augmenting effect upon adrenals of hypophysectomized rats (23).

Corticotropin has also been suspected as a possible etiologic factor in bilateral adrenal hyperplasia. Although some workers have claimed to find corticotropin in the plasma of such patients (24, 25) most observers have failed to detect increased amounts of this substance by a variety of assay procedures (4–7). Furthermore, administration of the usual commercial preparations of ACTH has not been shown to cause the degree of adrenal cortical hyper-response found in patients with adrenal hyperplasia (8, 9).

[5] Average plasma 17-OH-corticosteroid levels in 15 patients with bilateral adrenal hyperplasia, 29 micrograms per 100 ml.; average level in six patients with adrenal cortical tumor, 43 micrograms per 100 ml. (9).

Since it seems unlikely that either adrenal steroids or ACTH can account for the observed adrenal weight-maintaining activity, the question of a possible role of other hormones may be raised. Liddle, Island, Rinfret, and Forsham (26) studied a material prepared by Rinfret from equine pituitaries which exhibited adrenal weight-maintaining activity and was capable of enhancing the sensitivity of the human adrenal cortex to "orthodox" ACTH, as measured by excretion of urinary corticosteroids. This equine pituitary extract in itself had very little steroidogenic effect. It is not known whether or not the weight-maintaining "substance" in plasma of patients with adrenal hyperplasia has the property of enhancing the steroidogenic effect of ordinary ACTH upon the adrenal cortex.

Adrenal weight-maintaining effects have been found in growth hormone fractions by Reinhardt, Geschwind, and Li (10), Young and his associates (11), and Cater and Stack-Dunne (12). In the study reported here, growth hormone showed partial adrenal weight-maintaining activity as did the plasma of two patients with acromegaly in whose blood one might expect to find excessive somatotropin (27). It will be recalled that these two acromegalic patients did not show adrenal cortical hyper-response to ACTH.

The finding of adrenal weight-maintaining activity in the plasma of pregnant women in the third trimester is of interest in view of the alleged hyperresponsiveness of the adrenal cortex (2). The relationship, if any, between the two observations is not clear. It may perhaps be relevant that increased growth hormone levels have been found by Contopoulos and Simpson (28) in blood of pregnant rats, and that Cater and Stack-Dunne (12) have detected adrenal weight-maintaining activity in human placental extracts. No claim can be made on the basis of the data presented that either the adrenal hypersensitivity or the adrenal weight-maintaining activity in human pregnancy blood is or is not related to growth hormone. Furthermore, with our limited knowledge, it is impossible to decide at this time whether the adrenal weight-maintaining activity found in Cushing's syndrome with adrenal hyperplasia, acromegaly, and pregnancy is due to the same substance or to different substances.

SUMMARY

1. Adrenal weight-maintaining activity was detected in plasma of patients with Cushing's syndrome due to bilateral adrenal hyperplasia. The assay used was the prevention of complete adrenal atrophy in hypophysectomized rats. No adrenal weight-maintaining activity was found in plasma of three patients with Cushing's syndrome due to adrenal tumor, in normal subjects, or in a pool of plasma from patients with primary adrenal cortical insufficiency.

2. Adrenal weight-maintaining activity was observed in three pools of plasma from women in the third trimester of pregnancy and in two patients with active acromegaly.

3. On the basis of this study, no statement can be made concerning the nature or origin of the substance or substances responsible for adrenal weight-maintaining activity in bilateral adrenal hyperplasia, late pregnancy, or active acromegaly.

ACKNOWLEDGMENTS

The authors wish to express their thanks to Dr. George Sayers for his suggestions concerning this study. They also are deeply grateful to Dr. John W. Fertig, Professor of Biostatistics, Columbia University, and his associates for their invaluable help in the analysis of the data. Miss Myra Feder performed able technical assistance.

REFERENCES

1. Grumbach, M. M., Bongiovanni, A. M., Eberlein, W. R., van Wyk, J. J., and Wilkins, L., Cushing's syndrome with bilateral adrenal hyperplasia: A study of the plasma 17-hydroxycorticosteroids and the response to ACTH. Bull. Johns Hopkins Hosp., 1955, **96**, 116.
2. Christy, N. P., Wallace, E. Z., and Jailer, J. W., The effect of intravenously-administered ACTH on plasma 17, 21-dihydroxy-20-ketosteroids in normal individuals and in patients with disorders of the adrenal cortex. J. Clin. Invest., 1955, **34**, 899.
3. Lindsay, A. E., Migeon, C. J., Nugent, C. A., and Brown, H., The diagnostic value of plasma and urinary 17-hydroxycorticosteroid determinations in Cushing's syndrome. Am. J. Med., 1956, **20**, 15.
4. Taylor, A. B., Albert, A., and Sprague, R. G., Adrenotrophic activity of human blood. Endocrinology, 1949, **45**, 335.
5. Sydnor, K. L., Sayers, G., Brown, H., and Tyler, F. H., Preliminary studies on blood ACTH in man. J. Clin. Endocrinol. & Metab., 1953, **13**, 891.
6. Paris, J., Upson, M., Jr., Sprague, R. G., Salassa, R. M., and Albert, A., Corticotropic activity of

human blood. J. Clin. Endocrinol. & Metab., 1954, 14, 597.

7. Bethune, J. E., and Nelson, D. H., Personal communication.
8. Bayliss, R. I. S., and Steinbeck, A. W., The adrenal response to corticotrophin—effect of ACTH on plasma steroid levels. Brit. M. J., 1954, 1, 486.
9. Christy, N. P., Longson, D., and Jailer, J. W., Studies in Cushing's syndrome. I. Observations on the response of plasma 17-OH-corticosteroid levels to corticotropin. Am. J. Med., 1957, In press.
10. Reinhardt, W. O., Geschwind, I. I., and Li, C. H., On the evidence suggesting a multiplicity of adrenocorticotrophic hormones. Acta endocrinol., 1951, 8, 393.
11. Young, F. G., ACTH—a single substance or a mixture of hormones *in* Adrenal Cortex, Transactions of the Fifth Conference, Josiah Macy, Jr. Foundation. New York, Corlies, Macy & Company, Inc., 1954, p. 97.
12. Cater, D. B., and Stack-Dunne, M. P., Mitotic activity in the adrenal cortex studied in the rat *in* Ciba Foundation Colloquia on Endocrinology. London, J. & A. Churchill Ltd., 1955, 8, 31.
13. Simpson, M. E., Evans, H. M., and Li, C. H., Bioassay of adrenocorticotropic hormone. Endocrinology, 1943, 33, 261.
14. Sayers, G., Blood ACTH. J. Clin. Endocrinol. & Metab., 1955, 15, 754.
15. Fujita, T., Determination of corticotropin (ACTH) in human blood and urine by a modified oxycellulose method. J. Clin. Endocrinol. & Metab., 1957, 17, 512.
16. Munson, P. L., Barry, A. G., Jr., and Koch, F. C., A simplified hypophysectomized rat adrenal ascorbic acid bioassay method for adrenocorticotropin (ACTH): Specificity and application to preparative problems. J. Clin. Endocrinol., 1948, 8, 586.
17. Sayers, M. A., Sayers, G., and Woodbury, L. A., The assay of adrenocorticotrophic hormone by the adrenal ascorbic acid-depletion method. Endocrinology, 1948, 42, 379.
18. Bethune, J. E., Ganong, W. F., Hume, D. M., and Nelson, D. H., Plasma levels of ACTH in normal subjects and in Addisonian patients following corticosteroid administration. J. Clin. Endocrinol. & Metab., 1956, 16, 913.
19. Migeon, C. J., Bertrand, J., and Wall, P. E., Physiological disposition of 4-C^{14}-cortisol during late pregnancy. J. Clin. Invest., 1957, 36, 1350.
20. Christy, N. P., and Jailer, J. W., Unpublished observations.
21. Saffran, M., and Schally, A. V., *In vitro* bioassay of corticotropin: Modification and statistical treatment. Endocrinology, 1955, 56, 523.
22. Winter, C. A., Hollings, H. L., and Stebbins, R. B., The effect of androgenic hormones upon the adrenal atrophy produced by cortisone injections and upon the anti-inflammatory action of cortisone. Endocrinology, 1953, 52, 123.
23. Gaunt, R., Tuthill, C. H., Antonchak, N., and Leathem, J. H., Antagonists to cortisone: An ACTH-like action of steroids. Endocrinology, 1953, 52, 407.
24. Gray, C. H., and Parrott, D. M. V., Observations on a method of measuring adrenocorticotrophic hormone in plasma. J. Endocrinology, 1953, 9, 236.
25. Sulman, F. G., Chromatophorotropic activity of human blood: Review of 1200 cases. J. Clin. Endocrinol. & Metab., 1956, 16, 755.
26. Liddle, G. W., Island, D., Rinfret, A. P., and Forsham, P. H., Factors enhancing the response of the human adrenal to corticotropin: Is there an adrenal growth factor? J. Clin. Endocrinol. & Metab., 1954, 14, 839.
27. Segaloff, A., Konrad, E. L., Flores, A., Segaloff, Ann, and Hardesty, M., The growth hormone content of human plasma. Endocrinology, 1955, 57, 527.
28. Contopoulos, A. N., and Simpson, M. E., Increased growth hormone activity in plasma of pregnant rats. Federation Proc., 1956, 15, 39.
29. Fisher, R. A., Statistical Methods for Research Workers, 11th ed. Edinburgh, Oliver & Boyd, Ltd., 1950, p. 122.