Journal of Clinical Investigation
Vol. 46, No. 2, 1967

ACTH Antibodies in Patients Receiving Depot Porcine ACTH to Hasten Recovery from Pituitary-Adrenal Suppression *

NORMAN FLEISCHER, KAORU ABE, GRANT W. LIDDLE,† DAVID N. ORTH, AND WENDELL E. NICHOLSON

(From the Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tenn.)

Summary. Six patients who had experienced prolonged steroid-induced pituitary-adrenal suppression were treated with 100 U of depot porcine ACTH every 2 to 4 days for several months. Such treatment did not hasten the recovery of normal pituitary-adrenal function compared with the rate of recovery of a group of similarly suppressed patients who received no depot ACTH. Eight of nine patients who received prolonged courses of depot porcine ACTH developed antibodies to ACTH that cross-reacted with endogenous ACTH, binding it in the circulation in inactive form and retarding its removal from the circulation. The presence of such antibodies did not in itself grossly alter pituitary-adrenal interrelationships.

Introduction

After prolonged and profound suppression of human pituitary-adrenal function by cortisol-like steroids, there is frequently a delay of several months before normal function is regained (1). The course of recovery is characterized by an initial phase in which both adrenocorticotropic hormone and cortisol concentrations in the blood are subnormal. Then there is an intermediate phase during which ACTH increases to concentrations that are normal or greater, but adrenocortical responsiveness to ACTH remains subnormal. In the final phase, normal adrenocortical responsiveness is regained, so that cortisol is secreted in normal quantities in response to normal concentrations of ACTH in the plasma.

The present study concerned six patients who had experienced profound pituitary-adrenal suppression and who were then treated intermittently with depot preparations of porcine ACTH in an effort to hasten their recovery of adrenocortical responsiveness. Unfortunately, this treatment did not shorten the period of pituitary-adrenal recovery, but in the course of the treatment we observed that all six patients developed neutralizing antibodies to porcine ACTH. These antibodies cross-reacted with endogenous human ACTH, binding it in the circulation in inactive form.

Studies were performed, first, to characterize the antibodies and, second, to determine whether their presence in the circulation seriously modified pituitary-adrenal function. For the latter purpose, additional observations were made in three patients who had received repeated injections of depot porcine ACTH without having undergone prior pituitary-adrenal suppression. Two of these patients were also found to have antibodies to ACTH.

* Submitted for publication July 11, 1966; accepted October 20, 1966.

These studies were supported in part by the following grants-in-aid from the National Institutes of Health of the U. S. Public Health Service: 5-K6-AM-3782, 8-MOI-FR-95, T1-AM-5092, and 5-ROI-AM-05318.

† Address requests for reprints to Dr. Grant W. Liddle, Vanderbilt University School of Medicine, Nashville, Tenn. 37203.

Methods

Clinical. The principal group of patients were six individuals who had experienced continuous pituitary-adrenal suppression for at least 1 year before study. In four patients the suppression was the result of cortisol secretion by an adrenocortical tumor. The other two had

been treated with supraphysiological doses of cortisol analogues for rheumatoid arthritis. The degree of corticosteroid excess was sufficient to induce Cushing's syndrome in all six patients. The degree and duration of pituitary-adrenal suppression were comparable to those of the larger group previously reported from this laboratory by Graber and associates (1). Correction of the cortisol excess was achieved either by removal of the adrenocortical tumor or discontinuation of the exogenous steroid. Withdrawal of corticosteroids was accomplished in all cases over a period of 1 to 4 weeks. The course of pituitary-adrenal recovery was dated from the time the last dose of corticosteroid was administered. The six patients were given 100 U of depot porcine ACTH [1] intramuscularly every 2 to 4 days for periods of 2 to 22 months. The speed of recovery was compared with that of the earlier study in which ACTH was not used.

Three patients without prior endocrine disease who had never undergone pituitary-adrenal suppression were studied for circulating antibodies to ACTH after having received intermittent injections of depot porcine ACTH for periods of 5 months to 10 years.

Twenty-nine patients with elevated levels of plasma ACTH who had never received courses of exogenous ACTH and 20 normal subjects were also studied for evidence of circulating antibodies to ACTH.

Laboratory. Plasma ACTH levels were measured by the method of Lipscomb and Nelson (2) utilizing the amount of corticosterone in the adrenal effluent of hypophysectomized rats as the index of ACTH activity. Specimens of plasma to be assayed were divided into two portions. One was assayed without further manipulation. The other was acidified before assay, either by the addition of HCl to adjust the pH to 2.0 or by extraction at acid pH as previously described (3). In patients receiving exogenous ACTH, blood was drawn for ACTH and 17-hydroxycorticosteroid determinations 1 week or more after the last injection of ACTH.

The presence of neutralizing antibodies to ACTH was tested by a method previously described (4), in which known quantities of standard ACTH [2] were incubated in "immune" serum diluted 1:5 or greater for 30 minutes before assay. The adrenal-stimulating potency of ACTH incubated in this manner was compared with that of ACTH incubated in normal human serum identically diluted for 30 minutes before assay.

Binding of ^{131}I-labeled ACTH with antiserum was tested by the chromatoelectrophoretic method of Yalow, Glick, Roth, and Berson (5), using porcine ACTH [3] with a potency of 100 IU per mg by the assay method of Lipscomb and Nelson.

[1] Zinc corticotropin (Organon, Inc., West Orange, N. J.) or Acthar gel (Armour Pharmaceutical Co., Kankakee, Ill.).

[2] The porcine ACTH used in these tests was the Third International Standard Corticotropin. The human ACTH was an oxycellulose preparation, 10 IU per mg, kindly provided by Dr. Maurice Raben.

[3] Prepared by Mann Research Laboratories, Inc., New York, N. Y.

Plasma (6) and urinary (7) 17-hydroxycorticosteroids (17-OHCS) were determined by modifications of the method of Silber and Porter (8).

Results

Lack of effect of depot porcine ACTH therapy on the rate of recovery from chronic pituitary-adrenal suppression

Since previous studies had indicated that adrenal recovery lags behind pituitary recovery after prolonged and profound steroid-induced suppression (1), we attempted in the present study to hasten adrenal recovery by administering exogenous ACTH in depot form every 2 to 4 days. From time to time, we interrupted treatment with exogenous ACTH in order to evaluate the adequacy of endogenous pituitary-adrenal function. We found that plasma 17-OHCS values did not return to normal more rapidly in the six patients who received ACTH during the present study than in comparable patients previously studied who did not receive ACTH (Figure 1). Urinary 17-OHCS also remained subnormal in both groups for a comparable period. On the basis of the available data, therefore, it is impossible to conclude that the administration of depot porcine ACTH every 2 to 4 days hastens the ultimate recovery from chronic pituitary-adrenal suppression.

Effect of treatment with depot porcine ACTH on endogenous plasma ACTH concentration

An unexpected consequence of treatment with depot porcine ACTH was a striking elevation of endogenous plasma ACTH concentrations (Figure 2). Previous studies had indicated that, during the third to fifth months of recovery from pituitary-adrenal suppression, endogenous ACTH concentrations in the plasma are frequently elevated in patients who have not received depot porcine ACTH (1). However, the degree of elevation was much more striking in the patients of the present study who had received courses of treatment with depot porcine ACTH.

The following evidence indicated that the ACTH in the plasma of the treated patients was endogenous. *1*) High concentrations of ACTH were found even though blood was not drawn for assay until 1 to 3 weeks after the last previous injection of porcine ACTH. *2*) ACTH disappeared from plasma only when pituitary secretion was suppressed with dexamethasone. When dexametha-

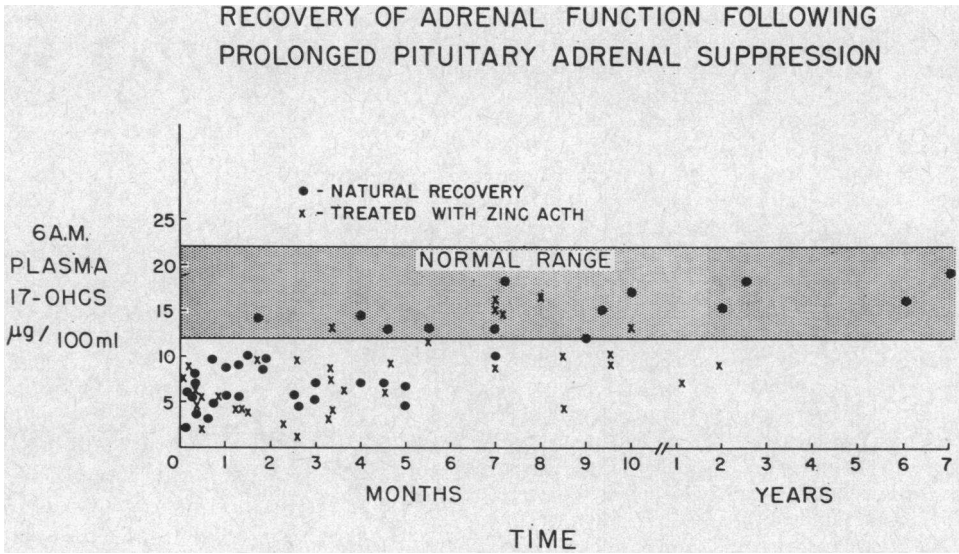

FIG. 1. PLASMA 17-HYDROXYCORTICOSTEROIDS (17-OHCS) DURING RECOVERY FROM STEROID-INDUCED PITUITARY-ADRENAL SUPPRESSION. Observations in patients treated with depot porcine ACTH are represented by X's. Observations in patients not treated with ACTH are represented by ●'s.

sone was discontinued, plasma ACTH returned to the previously elevated values without further administration of exogenous hormone (Figure 3).

Although plasma ACTH concentrations were much higher in patients who had received depot porcine ACTH than in those who had not, the plasma and urinary 17-OHCS were similar in the two groups. This suggested the possibility that much of the ACTH in the plasma of the ACTH-treated patients was circulating in an inactive form. This idea was corroborated by the further finding that adrenal secretory activity, although low in most of the patients, could be increased in all cases by giving infusions of exogenous ACTH. Previous studies (9) have indicated that concentrations of plasma ACTH slightly in excess of 3 mU per 100 ml will induce maximal adrenal secretory activity. Even though the ACTH content of the plasma of our patients ranged as high as 150 mU per 100 ml, their adrenal glands were not maximally stimulated. In the absence of exogenous ACTH the urinary 17-hydroxycorticosteroids of these patients ranged from 2 to 6 mg per day, but in response to 50 U of ACTH administered as a constant intravenous infusion for 8 hours, the urinary 17-hydroxycorticosteroids rose to 6 to 23 mg per day.

A possible explanation for the ineffectiveness of the high concentrations of endogenous ACTH in the plasma of our patients was derived from some previous studies of animals that had deliberately been immunized with ACTH. It had been observed that, in the presence of neutralizing antibodies, circulating ACTH could be present in high titers without stimulating adrenal secretion. By acidification of the plasma, however, the antibody-ACTH complexes could be dissociated, releasing biologically active ACTH. It was, therefore, postulated that our patients had been inadvertently immunized with ACTH. If so, then a large proportion of the ACTH present in their plasma could have been bound to ACTH antibodies in such a way that the ACTH was ineffective in stimulating their adrenal glands. The disproportionately high titers of ACTH in the plasma extracts that were injected into the rats employed in the assay procedure could be explained by the thesis that the antibodies had become dissociated from the ACTH by the process of acidification of the plasma in preparation for bioassay of the hormone. Confirmation of this postulate was obtained by assaying the plasma of our patients with and without prior acidification. The biologic activity of nonacidified plasma was much lower than

that of acidified samples in eight of the nine patients who had undergone treatment with depot porcine ACTH (Table I, group I).

It should be noted that acidification does not enhance plasma ACTH activity in the absence of ACTH antibodies. Thus, acidification had no effect on the ACTH potency of plasma from patients with untreated Addison's disease, patients who had previously undergone bilateral adrenalectomy for Cushing's disease, or patients with the ectopic ACTH syndrome (Table I, group II).

Characterization of ACTH antibodies in patients treated with depot porcine ACTH

Although the effects of acidification on the apparent ACTH content of plasma strongly suggested that the plasma of our patients contained neutralizing antibodies to ACTH, further documentation of this point seemed necessary. The following studies were performed to characterize these antibodies.

In vitro neutralization of ACTH. When ACTH

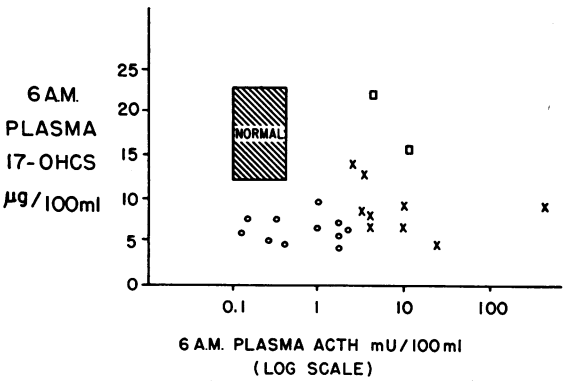

FIG. 2. COORDINATE PLASMA ACTH AND 17-OHCS LEVELS. Values for normal subjects are represented by the shaded rectangle. The unshaded rectangles represent patients with no adrenal abnormality who had received long courses of therapy with depot porcine ACTH. The other observations were made on patients during the third, fourth, or fifth month of recovery from prolonged profound steroid-induced pituitary-adrenal suppression. × = patients treated with depot porcine ACTH. ○ = patients not treated with ACTH. All ACTH assays were performed after acid extraction of plasma.

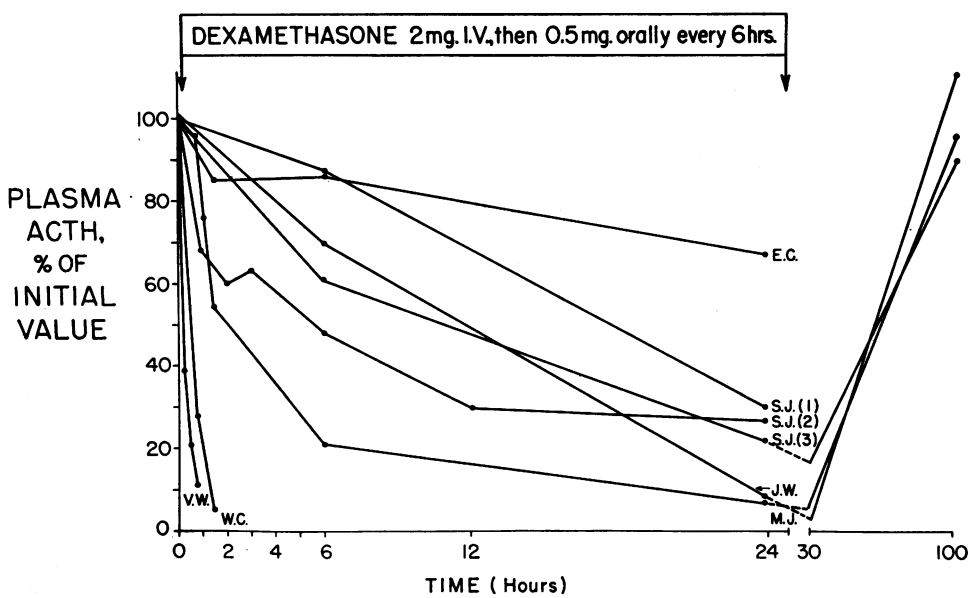

FIG. 3. RATES OF DISAPPEARANCE OF ENDOGENOUS TOTAL PLASMA ACTH. Serial determinations of total plasma ACTH were made on four patients with ACTH antibodies (S.J., E.C., M.J., J.W.), one Addisonian patient (V.W.), and one normal subject treated with metyrapone, 250 mg per hour for 36 hours (W.C.). Patient S.J. was studied on three separate occasions. We used dexamethasone to suppress ACTH secretion in order to simplify the study of plasma ACTH disappearance rates. The initial concentrations of ACTH in the plasma, in milliunits per 100 ml, were as follows: S.J. (1) 39, S.J. (2) 73, S.J. (3) 33, E.C. 2.7, M.J. 2.6, J.W. 2.7, V.W. 7.4, and W.C. 1.5.

TABLE I

*Plasma ACTH concentrations of patients who had previously been treated with depot porcine ACTH (group I) and patients who had not received depot porcine ACTH (group II)**

Group I			Group II		
	ACTH in unaltered plasma	ACTH in acidified plasma		ACTH in unaltered plasma	ACTH in acidified plasma
	mU/100 ml			*mU/100 ml*	
			Cushing's disease postadrenalectomy		
1 M.J.	<1.2	2.3	1	1.9	1.3
	<1.2	2.6	2	2.0	1.4
	<1.2	4.0	3	5.0	2.4
	<1.2	3.1	4	1.1	0.6
			5	18.0	20.0
2 E.C.	<1.0	2.6	6	13.0	21.0
	<1.0	2.7	7	6.4	4.8
			8	1.5	1.5
3 S.J.	2.3	39.0	9	1.9	2.1
	1.5	33.0	10	1.1	1.2
	3.0	60.0	11	1.7	1.5
	2.2	164.0	12	10.0	7.0
	6.0	118.0	13	1.4	1.8
	4.4	116.0	14	2.1	4.3
	9.9	72.0	15	1.9	1.1
	8.6	160.0	16	1.7	1.1
	5.2	69.0	17	5.2	5.0
	8.4	57.0	Addison's disease		
			18	2.6	3.3
4 J.W.	<1.2	2.1	19	3.6	3.6
	<1.2	3.1	20	2.5	1.7
	3.5	12.0	21	2.9	3.0
	1.9	16.0	22	1.3	1.5
	1.5	7.9	23	4.6	6.0
	3.1	19.0	24	1.3	1.5
	6.0	20.0	25	1.0	1.2
			26	1.1	0.8
5 L.T.	<1.0	7.0	27	1.6	1.6
			Ectopic ACTH syndrome		
6 R.K.	<1.0	10.0			
			28	1.6	1.6
7 M.S.	<1.0	5.0	29	1.4	1.4
8 F.S.	<1.0	4.0	Average, group II	3.5	3.6
9 G.F.	<1.0	0.4			

* Paired assays were performed on the same plasma samples. Serial studies were performed in some patients in group I over periods of several months.

is incubated with specific ACTH antibodies, it loses biologic potency. The neutralization of ACTH can be reversed by acidification of the hormone-antiserum mixtures, indicating that the antiserum inactivates ACTH by binding it and not by destroying it (4). Sera of four patients (J.W., M.J., S.J., E.C.) who had been treated with depot porcine ACTH and sera of 20 normal subjects were tested for neutralizing antibodies to ACTH in the following manner. Known quantities of ACTH were incubated at 37° C at pH 7 in phosphate buffer (4) or in serum for 30 minutes. The adrenal-stimulating potency of each mixture was then tested by measuring its effect on corticosterone secretion in the hypophysectomized rat. Sera from normal subjects diluted 1 : 5 or greater and incubated with ACTH for just 30 minutes had no appreciable effect on the biologic potency of ACTH. That is to say, ACTH treated in this way had approximately the same potency as did ACTH incubated in phosphate buffer for 30 minutes. In contrast, serum from each of the four patients neutralized the biologic activity of both porcine and human ACTH. An example is illustrated in Figure 4. In each case the neutralization of ACTH activity could be reversed by acidification of the hormone-antiserum mixture. These observations can be interpreted

to mean that the four patients had developed neutralizing antibodies in response to repeated injections of the foreign polypeptide, porcine ACTH, and that the antibodies thus formed were capable of cross-reacting with human ACTH, both *in vivo* and *in vitro*. Such cross-reactions have been observed before (4, 5) and are not surprising, since human and porcine ACTH are thought to differ from each other in only 3 of their 39 amino acids (10).

Binding of ACTH-^{131}I. When ^{131}I-labeled ACTH is bound to specific ACTH antibodies, it can be separated from unbound or "free" ACTH-^{131}I by chromatoelectrophoresis. The specificity of this reaction can be verified by showing that ACTH-^{131}I can be displaced from the "bound" to the free fraction by the addition of a small excess of pure unlabeled ACTH but not by other proteins or polypeptides (Figure 5). Sera of seven patients (F.S., J.W., R.K., M.J., S.J., E.C., and G.F.) who had been treated with depot porcine ACTH and sera of 20 normal subjects were tested for their capacity to bind ACTH-^{131}I. Six of the seven had sera that bound ACTH-^{131}I so that it migrated with the globulins during subsequent chromatoelectrophoresis. In all six patients the ACTH-^{131}I could be displaced from the bound to the free fraction by the addition of 0.05 μg of unlabeled ACTH. Patient G.F., whose serum did not bind ACTH-^{131}I, was the one patient without evidence of *in vivo* binding of endogenous ACTH (Table I, group I). In no instance did serum from a normal subject bind ACTH-^{131}I.

Electrophoretic mobility of ACTH antibodies. In order to determine whether the ACTH-neutralizing and ACTH-^{131}I-binding properties of immune sera resided in the γ-globulin fraction (as would be expected if they were properties of specific antibodies), we subjected 0.5 ml of serum of patient S.J. to paper electrophoresis. The electrophoretic strips were then cut into portions corresponding to the albumin, α-globulin, and β- plus γ-globulin fractions. The proteins were eluted from the paper with a phosphate buffer containing 0.5% bovine globulin, and the fractions were tested for their capacity to neutralize the biologic activity of ACTH and to bind ACTH-^{131}I. Only the β- plus γ-globulin fraction had appreciable ACTH-neutralizing or ACTH-^{131}I-binding capacity (Table II).

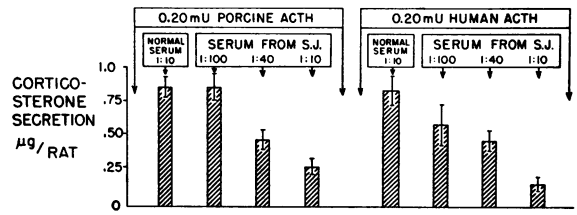

FIG. 4. NEUTRALIZATION OF ACTH BY HUMAN ANTISERUM. In all experiments each rat received 0.20 mU of porcine or human ACTH. The height of each bar represents the mean corticosterone secretion induced in hypophysectomized rats by the ACTH. The brackets represent standard errors of the means. In control experiments the ACTH was incubated in normal serum. In the other experiments the ACTH was incubated in serum obtained from patient S.J. in the dilution shown above each bar. We gave this patient dexamethasone for 5 days before the time her serum was obtained to reduce the serum ACTH content to negligible values.

TABLE II

Neutralizing and binding capacity of protein fractions of serum from a patient (S.J.) with ACTH antibodies

Serum fraction incubated with ACTH	Neutralization of ACTH (bioassay): left adrenal vein corticosterone	Binding of ACTH (radioimmunoassay): bound/free ACTH-^{131}I
	μg/3 min	
Paper-buffer control	0.92 ±0.20*	0.00
Albumin fraction	0.72 ±0.14	0.01
α-globulin fraction	0.82 ±0.19	0.05
β- and γ-globulin fraction	0.29 ±0.10	1.32
Paper-buffer control incubated with no ACTH	0.05 ±0.005	

* Mean ± standard error.

Effects of temperature. The ACTH-neutralizing capacity of immune sera was not appreciably affected by prior heating at 56° C for 36 minutes, indicating that complement is not essential for the *in vitro* reaction of antiserum with ACTH (11). Immune sera were effective in neutralizing ACTH whether incubation was carried out at 37 or 4° C.

Pituitary-adrenal dynamics in the presence of ACTH antibodies

Subsequent discussion will utilize the terms "free ACTH" to denote that detectable in unacidified plasma and "total ACTH" to indicate that detectable in acidified or acid-extracted samples of plasma. As indicated in Table I, group II, in the absence of ACTH antibodies, free ACTH and total ACTH are probably identical.

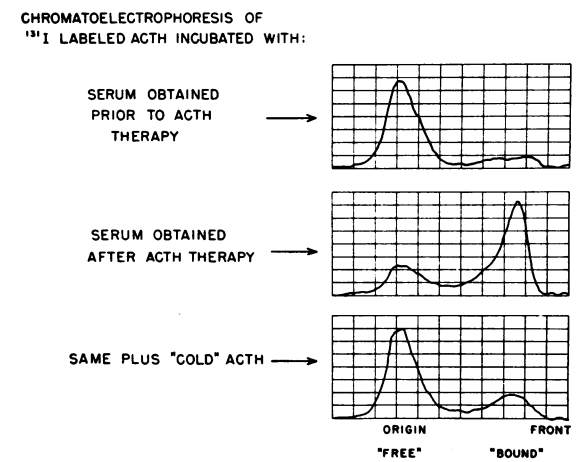

FIG. 5. CHROMATOELECTROPHORESIS OF ^{131}I-LABELED ACTH INCUBATED WITH SERUM FROM A PATIENT WITH CHRONIC STEROID-INDUCED PITUITARY-ADRENAL SUPPRESSION BEFORE AND AFTER SEVERAL MONTHS OF TREATMENT WITH ZINC PORCINE ACTH. The lowest panel shows a study with the same serum as that shown in the middle panel and illustrates the fact that the addition of 0.05 μg of unlabeled (cold) ACTH to the incubation mixture displaced the labeled hormone from the globulin fraction so that it remained at the origin with free ACTH.

Rate of disappearance of plasma total ACTH. Several experiments were performed to determine the relative rates of disappearance of total ACTH from the plasma of patients with neutralizing antibodies and of those without such antibodies (Figure 3). Of the two patients without antibodies, one had high plasma ACTH concentrations because of untreated Addison's disease, the other because of treatment with 250 mg of metyrapone [4] per hour for the preceding 36 hours. When 2 mg of dexamethasone phosphate was administered intravenously to these individuals, ACTH disappeared rapidly from their plasma with "half-times" of several minutes. In contrast, four patients (six studies) with high concentrations of bound ACTH in their plasma responded to dexamethasone (2 mg intravenously, followed by 1 mg orally every 6 hours) with very slow decreases in plasma ACTH concentration, with half-times of several hours. After dexamethasone was discontinued, ACTH concentrations returned to predexamethasone values, confirming the view that the ACTH was endogenous.

Lack of diurnal rhythm in total ACTH. Total ACTH concentrations in the plasma of patients with ACTH antibodies failed to exhibit a diurnal variation (Figure 6). This lack of an obvious diurnal rhythm in total ACTH concentrations stands in contrast to what has been observed in normal subjects and in cortisol-deficient patients who have not received depot porcine ACTH (1, 12).

Diurnal rhythm in plasma 17-OHCS and free ACTH. Patients with ACTH antibodies did exhibit a diurnal variation in plasma 17-OHCS (Table III). In the two patients (S.J. and J.W.) in whom free ACTH was detectable, there was a consistent diurnal variation in both free ACTH and plasma 17-OHCS. This would suggest that adrenal steroidogenesis *in vivo* is dependent on the level of free rather than total ACTH and that the level of free ACTH is not a constant function of the level of total ACTH.

Pituitary-adrenal function in nonsuppressed patients with ACTH antibodies. The possibility that the presence of ACTH antibodies might seriously impair pituitary-adrenal function was difficult to evaluate in patients convalescing from prolonged pituitary-adrenal suppression, because adrenal responsiveness was subnormal in that situation. Therefore, studies were performed in two patients (E.C. and F.S.) who had received intermittent injections of depot porcine ACTH over long periods but who had never received suppressive doses of corticosteroids. Free ACTH was not detectable in their plasma (less than 1 mU per 100 ml). Plasma and urinary 17-OHCS values were normal and were greater in the morning than in the evening. Adrenocortical responses to intravenous infusions of 50 U of ACTH of 8 hours' duration were normal. Pituitary-

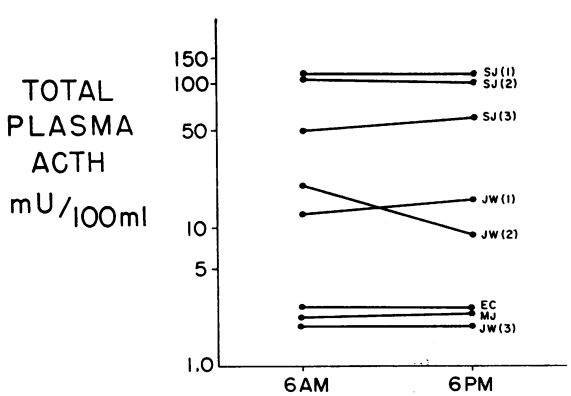

FIG. 6. LACK OF DIURNAL VARIATION IN TOTAL PLASMA ACTH CONCENTRATION IN PATIENTS WITH ACTH ANTIBODIES. Patients S.J. and J.W. were studied repeatedly over periods of several months.

[4] Metopirone, Ciba Pharmaceutical Co., Summit, N. J.

TABLE III

*6 a.m. and 6 p.m. concentrations of free ACTH in the plasma at various times during recovery from chronic pituitary-adrenal suppression in two patients with antibodies to ACTH**

Patient	Days of recovery from suppression	Plasma free ACTH		Plasma 17-OHCS	
		6 a.m.	6 p.m.	6 a.m.	6 p.m.
		mU/100 ml		*μg/100 ml*	
S.J.	139	6.0	4.4	9.2	4.9
	226	11.2	3.8	16.0	
	227	8.6		11.0	
	286	8.4	5.2	15.3	9.3
J.W.	109	3.5	1.9	4.1	4.9
	165	8.1	5.0	11.5	7.3
	206		1.5		9.8
	207	3.1	2.7	15.0	10.0
E.C.				22.0	8.3
L.T.				14.0	3.9
R.K.				10.8	3.9

* Also shown are plasma 17-hydroxycorticosteroid (17-OHCS) levels in these two patients and in three additional patients who had ACTH antibodies but whose free ACTH concentrations were less than 1 mU per 100 ml and, therefore, could not be quantified with certainty.

adrenal responses to metyrapone and to dexamethasone were normal as judged from measurements of urinary 17-OHCS. Thus, despite the fact that these two patients had neutralizing antibodies in their plasma, high levels of bound ACTH, and prolonged disappearance times of total ACTH, they appeared to have grossly normal pituitary-adrenal interrelationships.

Discussion

It has been well documented that specific antibodies to ACTH can be produced in a variety of experimental animals given repeated injections of ACTH or ACTH-albumin conjugates (4, 13–16). Previous evidence of the antigenicity of ACTH in human subjects has been published in the form of reports of allergic reactions to impure preparations of ACTH (17–19). Although certain of these studies provided strong evidence for antibodies to pituitary substances, it was not possible to state whether the antibodies were formed in response to ACTH or to impurities in the ACTH preparations. None of our patients demonstrated clinical allergic manifestations when treated with ACTH.

The evidence that the patients of the present study developed specific antibodies to ACTH may be summarized as follows. *1*) Sera from these patients possessed the capacity to neutralize human and porcine ACTH *in vitro,* a property which resided in the β- plus γ-globulin fraction. *2*) Sera from these patients bound ACTH-^{131}I to the globulin fraction, and the bound ACTH-^{131}I could be displaced by the addition of a small excess of unlabeled ACTH but not by the addition of other polypeptides. *3*) Large amounts of ACTH were bound in inactive form in the plasma of these patients. *4*) Individuals who had not received repeated injections of depot porcine ACTH did not develop sera that would neutralize ACTH or bind ACTH-^{131}I.

The high incidence of antibodies to ACTH (eight of nine patients studied) is analogous to what has been reported in patients receiving insulin (20).

The binding of ACTH to antibody appears to protect it from *in vivo* destruction as judged by the prolonged plasma half-time of total ACTH. Circulating insulin that is bound to insulin antibodies also has a prolonged rate of disappearance from plasma (21).

In order to ascertain the effects of circulating ACTH antibodies on pituitary-adrenal function, it is necessary to consider the immunized patients in two groups and compare them with appropriate nonimmunized control subjects. One group, those who were convalescing from prolonged pituitary-adrenal suppression, were similar to the suppressed nonimmunized patients reported previously in passing through three stages of recovery, the second of which was characterized by elevated concentrations of free ACTH in the plasma (1). They were also similar in their sluggish recovery

of adrenal responsiveness and in their exhibition of a diurnal variation in free ACTH and in plasma 17-OHCS with higher levels in the morning than in the evening. This group differed from their nonimmunized counterparts in having a greatly delayed rate of disappearance of total ACTH from plasma. The other group, those who had been immunized to ACTH without previously undergoing pituitary-adrenal suppression, differed from normal subjects in having a greatly delayed rate of disappearance of total ACTH from the plasma but resembled normal subjects in exhibiting definite diurnal variations in plasma 17-OHCS, in responding with increased excretion of 17-OHCS when treated with ACTH or with metyrapone, and in responding with decreased excretion of 17-OHCS when treated with dexamethasone. It must be concluded that the mere presence of ACTH antibodies does not grossly alter pituitary-adrenal interrelationships even though subtle changes can be demonstrated.

Although the presence of ACTH antibodies may not constitute a significant physiological problem for the patient, it does pose potential problems for the clinical physiologist who assays ACTH, whether the assays are performed by biological or immunological techniques.

References

1. Graber, A. L., R. L. Ney, W. E. Nicholson, D. P. Island, and G. W. Liddle. Natural history of pituitary-adrenal recovery following long-term suppression with corticosteroids. J. clin. Endocr. 1965, **25**, 11.
2. Lipscomb, H. S., and D. H. Nelson. A sensitive biologic assay for ACTH. Endocrinology 1962, 71, 13.
3. Island, D. P., N. Shimizu, W. E. Nicholson, K. Abe, E. Ogata, and G. W. Liddle. A method for separating small quantities of MSH and ACTH with good recovery of each. J. clin. Endocr. 1965, **25**, 975.
4. Fleischer, N., J. R. Givens, K. Abe, W. E. Nicholson, and G. W. Liddle. Studies of ACTH antibodies and their reactions with inactive analogues of ACTH. Endocrinology 1966, **78**, 1067.
5. Yalow, R. S., S. M. Glick, J. Roth, and S. A. Berson. Radioimmunoassay of human plasma ACTH. J. clin. Endocr. 1964, **24**, 1219.
6. Peterson, R. E., A. Karrer, and S. L. Guerra. Evaluation of Silber-Porter procedure for determination of plasma hydrocortisone. Analyt. Chem. 1957, **29**, 144.
7. Liddle, G. W., J. E. Richard, and R. E. Peterson. An improved method for assaying the steroidogenic potency of ACTH. Endocrinology 1955, **57**, 594.
8. Silber, R. H., and C. C. Porter. The determination of 17, 21-dihydroxy-20-ketosteroids in urine and plasma. J. biol. Chem. 1954, **210**, 923.
9. Ney, R. L., N. Shimizu, W. E. Nicholson, D. P. Island, and G. W. Liddle. Correlation of plasma ACTH concentration with adrenocortical response in normal human subjects, surgical patients, and patients with Cushing's disease. J. clin. Invest. 1963, **42**, 1669.
10. Lee, T. H., A. B. Lerner, and V. Buettner-Janusch. On the structure of human corticotropin (adrenocorticotropic hormone). J. biol. Chem. 1961, **236**, 2970.
11. Kabat, E. A., and M. M. Mayer. Experimental Immunochemistry, 2nd ed. Springfield, Ill., Charles C Thomas, 1961, p. 159.
12. Graber, A. L., J. R. Givens, W. E. Nicholson, D. P. Island, and G. W. Liddle. Persistence of diurnal rhythmicity in plasma ACTH concentrations in cortisol-deficient patients. J. clin. Endocr. 1965, **25**, 804.
13. Chase, J. H. Serological studies on crystalline adrenocorticotrophic hormone: the production of anti-adrenocorticotrophin. Endocrinology 1949, **45**, 96.
14. Gordon, G. L. The development of a refractory state to adrenocorticotrophic hormone. Endocrinology 1949, **45**, 571.
15. McGarry, E. E., J. C. Beck, L. Ambe, and R. Nayak. I. Polypeptide hormones. Some studies with antisera to growth hormone, ACTH, and TSH. Recent Progr. Hormone Res. 1964, **20**, 1.
16. McGuire, J., R. McGill, S. Leeman, and T. Goodfriend. The experimental generation of antibodies to α-melanocyte stimulating hormone and adrenocorticotropic hormone. J. clin. Invest. 1965, **44**, 1672.
17. Rosenblum, A. H., and P. Rosenblum. Anaphylactic reactions to adrenocorticotropic hormone in children. J. Pediat. 1964, **64**, 387.
18. Buytendijk, H. J., and F. Maesen. Comparative skin tests with animal and synthetic corticotrophin in patients hypersensitive to animal corticotrophin. Acta. endocr. (Kbh.) 1964, **47**, 613.
19. Perkoff, G. T., B. V. Jager, and F. H. Tyler. Complications in the management of Cushing's syndrome including anaphylactic reaction to intravenous adrenocorticotropin after subtotal adrenalectomy. J. clin. Endocr. 1955, **15**, 362.
20. Yalow, R. S., and S. A. Berson. Immunologic aspects of insulin. Amer. J. Med. 1961, **31**, 882.
21. Berson, S. A., R. S. Yalow, A. Bauman, M. A. Rothschild, and K. Newerly. Insulin-I^{131} metabolism in human subjects: demonstration of insulin binding globulin in the circulation of insulin treated subjects. J. clin. Invest. 1956, **35**, 170.