CHANGES IN PLASMA LEVELS OF 17-HYDROXYCORTICOSTEROIDS DURING THE INTRAVENOUS ADMINISTRATION OF ACTH. I. A TEST OF ADRENOCORTICAL CAPACITY IN THE HUMAN [1]

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The recognition of the important role played by the adrenal cortex in normal physiology and its significance in withstanding "stressful situations" has led to the development of many tests to measure its functional state and capacity in the human. Most of these tests rely on measurements of phenomena which are dependent on the effect of adrenocortical secretions on some special metabolic process, and do not involve the direct measurement of adrenocortical steroids. The frequent discrepancies between the clinical picture and these indirect tests indicate that they are not always reliable indices of adrenal function.

Recently, Thorn, Goetz, Streeten, Dingman, and Arons (1) have devised a test for the quantitative estimation of adrenal function based on the determination of butanol-soluble 17,21-dihydroxy-20-ketosteroids in the urine. Adrenal capacity is estimated by the increase in urinary excretion of these compounds after intravenous administration of adrenocorticotrophic hormone (ACTH). While this technique is a considerable improvement over the indirect methods, measurements based on the urine have several disadvantages. If adrenal function or capacity must be determined over short periods of time, the technical difficulties of obtaining quantitative urine samples at frequent intervals are great. Alterations in renal function and steroid metabolism may also interfere with interpretation in terms of the internal hormonal environment. The development of a method for measuring 17-hydroxycorticosteroids in plasma (2) has made it possible to study adrenocortical function and its response to ACTH more directly.

In a previous paper from our laboratory the response of the adrenal cortex was measured after the rapid intravenous injection of a single dose of ACTH (3). It was stated in that paper that "the immediate injection of a single dose of ACTH was selected for its simplicity, with the reservation that such a technique, although valuable as a measure of the immediate ability of the gland to respond to stimulation, would be less significant in the assessment of the capacity of the gland to resynthesize and elaborate additional hormones, once available stores had been exhausted. A more prolonged stimulation, such as intravenous infusion of ACTH over a period of many hours, would test this aspect of glandular function more effectively." The present paper describes the effects of prolonged intravenous infusion of ACTH on plasma 17-hydroxycorticosteroid levels in a group of normal resting individuals. From these studies a standard method of testing adrenal cortical capacity has been developed.

METHODS

The studies were performed on 39 normal subjects (33 males, 6 females) and six selected patients with adrenal insufficiency. All tests were begun between 8 and 9 A.M. and all the subjects were in the fasting state. The ACTH was given by continuous intravenous infusion in 500 ml. of normal saline or 5 per cent glucose in water.

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No subject was studied more frequently than every ten days. Most of the ACTH tests were performed with the same lot number (K56409) of "crude" ACTH (Armour), 25 I.U. being given over 6 hours. Some of the tests were performed with other ACTH preparations. The H.P. ("High Potency") Armour preparation is obtained by passage through oxycellulose and countercurrent distribution. Wilson ACTH (lot number 58792) is obtained by oxycellulose passage, as is the preparation obtained from Dr. Astwood. The ACTH obtained from Dr. Li (lot number L2397BP) had an activity of 80 I.U. per mgm. Corticotropin "Nyco" (lot number 034060) was obtained from the pituitary glands of whales and prepared by Nyegaard and Co., Norway. A few tests were done with Upjohn's ACTH (lot number CB243ZF).

Plasma 17-hydroxycorticosteroids were determined by modification (4) of the method of Nelson and Samuels (2). It should be pointed out that this method determines cortical steroids with a 17,21-dihydroxy-20-ketone group and includes such compounds as hydrocortisone, cortisone, and Reichstein's substance S. The method determines the hydrogenated forms of these steroids but not their water soluble conjugates. Although many substances in plasma will give the Porter-Silber reaction the material present after chloroform extraction and chromatography on florosil appears to be mostly hydrocortisone, particularly after ACTH. A detailed discussion of this problem and possible interfering non-steroidal substances is beyond the scope of this report but the frequent occurrence of undetectable levels in patients with adrenal insufficiency, two examples of which are presented here, is presumptive evidence of its specificity. Certain data concerned with the specificity of the method have been published previously (5, 6). A detailed study of the recoveries obtained, the character of steroids in human and dog plasma giving the reaction, and the possibility of interfering non-steroidal substances in plasma is being prepared for publication. Eosinophil counts were done by the method of Randolph (7).

RESULTS

In order to test adrenocortical capacity, the amount of ACTH administered and the period of time during which such ACTH is given are of paramount importance. The effects of the intravenous infusion of different amounts of ACTH on the 17-hydroxycorticosteroid levels in the plasma of normal individuals are compared in Table I. The time of infusion was 6 hours in this series. As little as 1 I.U. and possibly 0.1 I.U. of ACTH had an effect in raising the levels of 17-hydroxycorticosteroids in the blood plasma. These increases were, however, submaximal. What appeared to be maximal stimulation of the adrenal cortex was induced by 15 to 25 I.U. of ACTH. There was no significant difference between the increase in plasma 17-hydroxycorticosteroids during the administration of 50 I.U. of ACTH and that during 25 I.U. (Tables I, II, and Figure 1). A dose of 25 I.U. of ACTH was, therefore, selected as giving maximal stimulation of the adrenal cortex as measured by the increase in plasma 17-hydroxycorticosteroid levels in 6 hours.

TABLE I

Comparison of the changes in the levels of plasma 17-hydroxycorticosteroids when different doses of ACTH were infused intravenously over a 6-hour period *

Subject	Amount of ACTH I.U.	Plasma 17-OH-CS levels γ/100 ml. 0 hr.	2 hrs.	4 hrs.	6 hrs.
A	1/10	4	17	17	21
A	25	5	23	27	30
A	25	8	30	43	50
B	1	8	15	20	22
B	10	11	24	32	29
B	25	6	27	32	40
B	25	7	20	26	30
C	15	10	30	40	46
C	25	12	26	32	40
C	25	4	21	22	23
D	50	5	20	32	38
D	25	8	30	36	48
E	50	17	36	39	40
E	25	11	34	36	45

* Tests were run at two to three different dose levels in the same individual.

A series of experiments was performed to determine the rate of infusion of ACTH which would give a maximal rate of increase in plasma 17-hydroxycorticosteroids. A total of 25 I.U. of ACTH was infused over periods of 2, 4, 6, and 8 hours. As seen in Table III, there was no significant difference in the rates of increase of 17-

TABLE II

Levels of 17-hydroxycorticosteroids during the intravenous infusion of 50 I.U. ACTH over 6 hours

Subject	Plasma 17-OH-CS levels γ/100 ml. 0 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.
D	5	20		32	38
M	11	40		50	63
E	17	36		39	40
O	14		30		40
P	10		33		42
Q	15		35		48
R	16				51
S	15				48
T	10				50
Mean	13				47
Mean, 25 I.U. (Figure 1)	10				42

TABLE III

The effects of 25 I.U. ACTH over different periods of time upon plasma levels of 17-hydroxycorticosteroids

Subject	Length of infusion (*hrs.*)	Plasma 17-OH-CS levels γ/100 ml. 0 hr.	2 hrs.	4 hrs.	6 hrs.	8 hrs.
M	2	20	29			
N	2	10	33			
O	4	5	26	37		
P	4	3	20	28		
Q	8	10	24	43	44	46
A	8	14	34	40	62	55
A	6	8	30	43	50	
A	6	5	23	27	30	
B	8	10	25	34	34	23
B	6	6	27	32	40	
B	6	7	20	26	30	
C	8	10	28	25	31	31
C	6	12	26	32	40	
C	6	4	21	22	23	

hydroxycorticosteroids in the plasma among the different rates of infusion. Furthermore, when the dose was spread over 8 hours, the level at the end of the infusion did not differ significantly from that at 6 hours.

The means and standard deviations of the response of the plasma 17-hydroxycorticosteroid levels to the intravenous infusion of 25 I.U. of ACTH over 6 hours in 39 normal resting subjects are shown in Figure 1. It will be observed that the standard deviation of values increases proportionately with the mean.

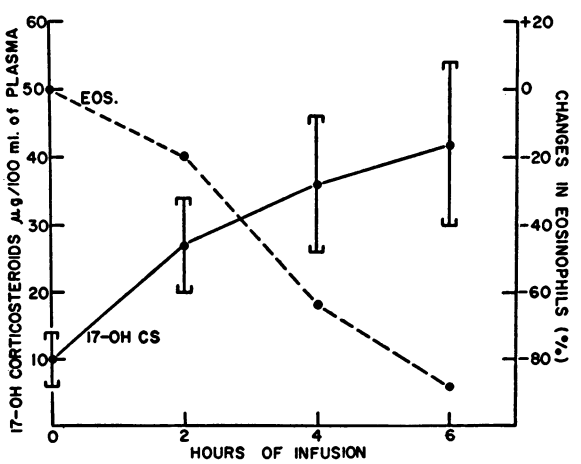

FIG. 1. PLASMA 17-HYDROXYCORTICOSTEROID LEVELS DURING THE INTRAVENOUS INFUSION OF 25 I.U. OF ACTH

The mean and standard deviation of the response of these levels in 39 normal subjects are shown. The changes in the eosinophilic leukocytes are correlated with the changes in the steroid levels.

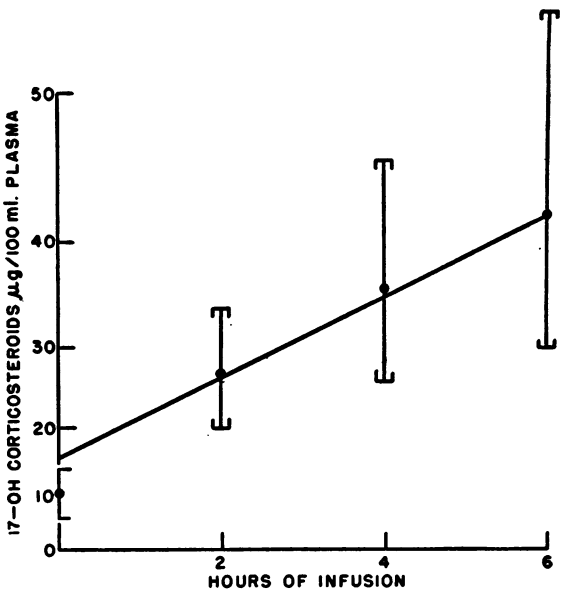

FIG. 2. THE MEAN AND STANDARD DEVIATION OF PLASMA 17-HYDROXYCORTICOSTEROID LEVELS DURING THE INFUSION OF 25 I.U. OF ACTH PLOTTED ON AN INVERSE LOGARITHMIC SCALE

In Figure 2 the rate of increase of plasma 17-hydroxycorticosteroids during the intravenous infusion of 25 I.U. of ACTH over 6 hours is plotted on an inverse logarithmic scale. Except for the initial 2-hour period, the mean values all fall along a straight line. This would be the result expected if there were constant input and removal proportionate to the level attained. In other work it has been demonstrated that once initial equilibrium has been achieved, 17-hydroxycorticosterone and cortisone are removed from the blood stream at a rate proportional to concentration (8, 9). During the first period, however, there is evidence of a more rapid increase. This may be due to small amounts of steroids which can be quickly mobilized for secretion. The 6-hour infusion permits the determination of three values at 2-hour intervals which are beyond this initial point, at which time a maximal constant rate of secretion seems to have been established. The shorter infusions were discarded because of possible confusion between initial output and that which could be maintained.

In Figure 3 is shown a representative curve of the plasma 17-hydroxycorticosteroids during and following the continuous infusion of ACTH over many hours. It is apparent that the levels reached

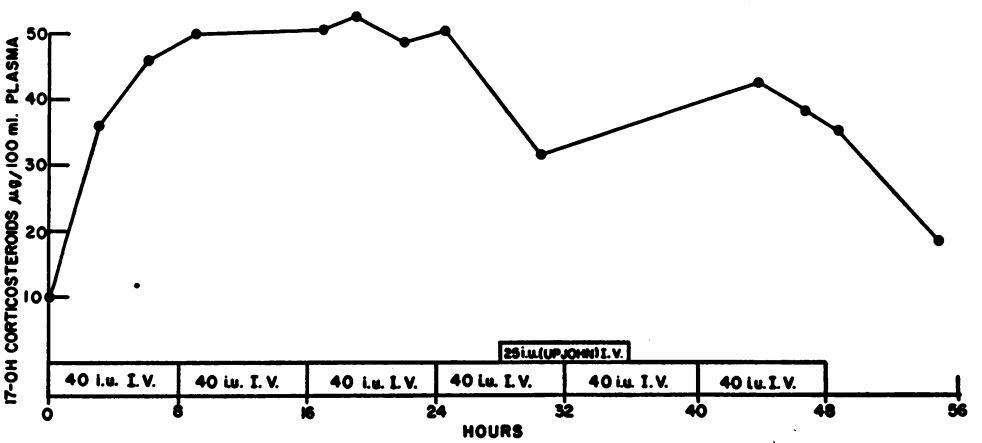

FIG. 3. PLASMA 17-HYDROXYCORTICOSTEROID LEVELS DURING THE CONTINUOUS INFUSION OF ACTH(40 I.U. WILSON ACTH q.8 H.) OVER 48 HOURS

Between the 28th and 36th hour an additional 25 I.U. of ACTH (Upjohn) was infused. The explanation of the apparent fall in level during this period is unkown at present.

a plateau after 6 hours which was maintained as long as the ACTH was given. The steroid levels returned to the normal range within 6 hours after cessation of the infusion. Halfway through the experiment the subject was given an additional 25 I.U. of ACTH (Upjohn) in addition to the continuous infusion that he was receiving. This was done in order to ascertain whether maximal adrenal stimulation was being obtained by the amount of ACTH which was given; if not, the additional ACTH should have raised the plasma 17-hydroxycorticosteroids further. Instead, the plasma sample at the end of this period gave a lower value.

It would appear, therefore, that the infusion of 25 to 30 I.U. in 6 hours achieves a stimulation of the adrenal cortex which is maximal. It is also a test of the capacity of the adrenal cortex to maintain production of cortical steroids having a 17,21-hydroxy-20-ketone group.

The response of the same subject to a standard ACTH test on different occasions showed considerable variation and is illustrated in Table IV. This variation in response does not seem to depend on the control level of plasma 17-hydroxycorticosteroids.

To determine whether the type of preparation would affect the test, six different preparations of ACTH were infused in the standard manner. It appeared that, in the dose given (25 I.U.), neither the animal source nor the difference in the technical procedures of preparing ACTH had any effect on its capacity to elicit maximal adrenocortical stimulation. Apparently, the U.S.P. assay is satisfactory for evaluation of the potency of ACTH administered intravenously in the human.

The validity of the procedure as a measure of adrenocortical capacity was tested in subjects with clinical symptoms of adrenal insufficiency (Figure 4). In each case there was little or no rise in the plasma 17-hydroxycorticosteroid levels fol-

TABLE IV

Plasma levels of 17-hydroxycorticosteroids following the intravenous infusion of 25 I.U. of ACTH over 6 hours in the same subject on different occasions

Subject	Plasma 17-OH-CS levels γ/100 ml.			
	0 hr.	2 hrs.	4 hrs.	6 hrs.
A	5	23	27	30
A	8	30	43	50
B	6	27	32	40
B	7	20	26	30
C	12	26	32	40
C	4	21	22	23
F	8	26	28	49
F	10	30	54	60
G	9	28	40	46
G	3	21	22	23
G	12	26	32	40
H	4	23	29	32
H	17	31	41	56
I	10	20	25	38
I	12	25	26	39
I	12	26	43	40
J	4	22	25	20
J	6	32	40	43
K	6	27	33	40
K	8	20	26	30
L	8	18	28	28
L	8	26	50	43

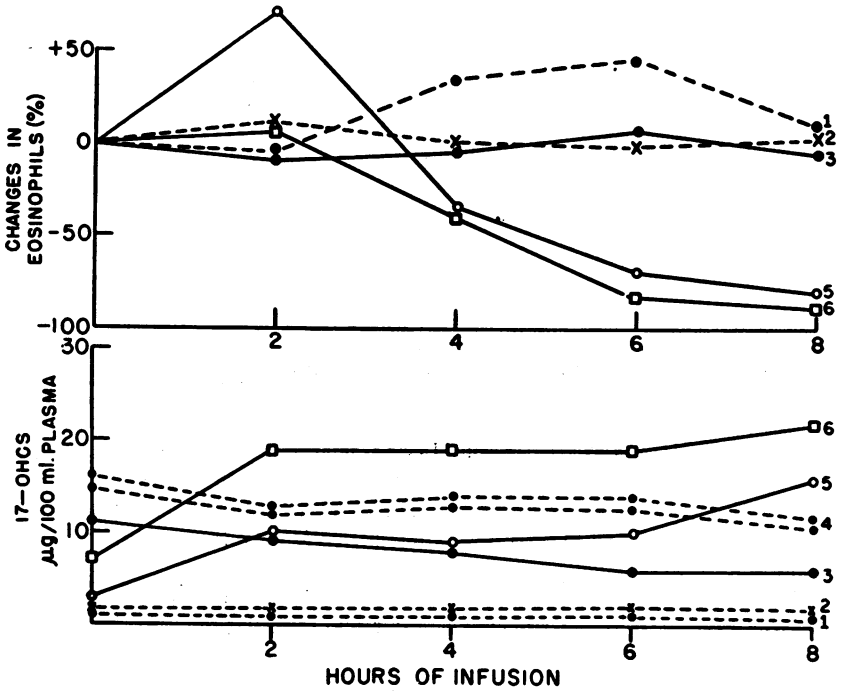

FIG. 4. CHANGES IN PLASMA 17-HYDROXYCORTICOSTEROID LEVELS AND CIRCULATING EOSINOPHILS IN SIX PATIENTS WITH ADRENAL INSUFFICIENCY DURING THE I.V. INFUSION OF 25 I.U. OF ACTH OVER 8 HOURS

lowing the intravenous infusion of 25 I.U. of ACTH over 6 to 8 hours. It is presumed that in the patients with demonstrable 17-hydroxycorticosteroid levels in plasma, the small amount of adrenal cortex still functioning was stimulated almost maximally by endogenous ACTH. No demonstrable steroid levels were seen after the standard ACTH test in Addisonian patients with no measurable plasma 17-hydroxycorticosteroid levels beforehand (Cases 1 and 2, Figure 4).

In each subject showing a significant rise in 17-hydroxycorticosteroid levels following ACTH administration, there was a definite effect on the levels of circulating eosinophils (Cases 5 and 6, Figure 1). Although the largest rise in 17-hydroxycorticosteroids occurred during the first 2 hours of ACTH infusion, a significant decrease in eosinophils of over 50 per cent did not occur until 4 hours after the beginning of the infusion (Figures 1, 4, and Table V). At the end of the infusion of ACTH the eosinophil count was lowest, while the plasma 17-hydroxycorticosteroids were usually highest. It is interesting to observe that in subjects with adrenocortical insufficiency a significant drop in the eosinophils was found only when the subject showed an increase of plasma 17-hydroxycorticosteroids following the standard test for adrenocortical capacity (Figure 4).

The usefulness of the response of the circulating eosinophils is, however, limited under "stressful" conditions. As can be seen from Table V, there were definite increases in the plasma levels of 17-hydroxycorticosteroids following the standard ACTH test in a case where the eosinophils were too low to give any indication of changes in corticosteroid levels.

TABLE V

Plasma 17-hydroxycorticosteroid levels and blood eosinophils following the administration of 25 I.U. of ACTH intravenously over 6 hours in a patient before and after a major surgical operation (removal of the gallbladder, general anesthesia)

Time after beginning of ACTH adm.	One week before surgery		Immediately after surgery	
	17-OH-CS in plasma *γ/100 ml.*	Eosinophils *per mm.*³	17-OH-CS in plasma *γ/100 ml.*	Eosinophils *per mm.*³
0	9	360	60	3
2	37	334	78	0
4	50	106	107	0
6	52	12	96	0

DISCUSSION

From the data presented it is evident that the intravenous administration of 25 I.U. ACTH over 6 hours serves as a test of adrenocortical capacity. The demonstration of diminished capacity by this test in patients who had shown symptoms of Addison's disease points to the reliability of the test. It is also apparent from the data that the increment in plasma 17-hydroxycorticosteroids during the first two hours may exceed the rate expected on the basis of constant input and logarithmic removal, and, therefore, probably includes release of readily mobilized precursor as well as maximal rate of synthesis. Continuation of the stimulation with ACTH beyond the 6-hour period results in little further elevation of 17-hydroxycorticosteroid levels and is, therefore, unnecessary for the evaluation of the adrenocortical capacity.

The test described offers some advantages over those presently in existence. The number of eosinophils cannot always be used as a reliable index of adrenocortical function since it is influenced by other factors (10). The urinary 17-ketosteroids arise from other sources besides the adrenal cortex. In addition, all measurements on urine involve the function of the kidneys. Abnormalities in these organs will influence the rate of excretion of steroids in relation to blood levels, and may, therefore, lead to erroneous results after ACTH stimulation.

In the procedure outlined here only the change in levels of free 17-hydroxycorticosteroids during ACTH administration is measured. The steroids excreted in the urine are largely conjugated metabolic products which are not determined in this method. Under these conditions if changes in the levels of conjugated compounds due to renal impairment occurred they would have little effect on the test. The present method, therefore, overcomes a number of errors involved in past procedures for measuring adrenal capacity. The amounts of blood required for the determination of plasma 17-hydroxycorticosteroids can be repeatedly withdrawn at short intervals. In addition, the administration of ACTH and its demands on the adrenal cortex can be evaluated concomitantly with the ACTH administration.

In conditions where the adrenocortical steroid environment is undergoing frequent changes, it is difficult to evaluate the influence of infused ACTH. Fluctuations in the amount of 17-hydroxycorticosteroids in the blood plasma may be due to endogenous changes rather than to the effect of exogenous ACTH. It is felt, therefore, that the test for adrenocortical capacity as described in this paper may best be applied in the normal resting human, in patients with adrenal insufficiency, and in pathological conditions where a stable adrenocortical environment has been established. Results under other circumstances must be interpreted with caution, and are of more qualitative than quantitative significance.

SUMMARY

1. Utilizing changes of 17-hydroxycorticosteroid levels in blood plasma following the intravenous administration of 25 I.U. ACTH over 6 hours, a test has been devised for evaluating adrenocortical capacity in the normal resting human.

2. When 50 I.U. ACTH were infused over the same period there was no significant increase in the rate of change of the plasma 17-hydroxycorticosteroids.

3. Individuals who showed symptoms of Addison's disease showed a decreased response even when the levels of 17-hydroxycorticosteroids before ACTH infusion were within normal limits.

4. Advantages and limitations of the test as a measure of adrenocortical capacity are discussed.

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