PLASMA CORTISOL RESPONSE TO SYNACTHEN (β^{1-24} CIBA) AT DIFFERENT TIMES OF THE DAY IN PATIENTS WITH RHEUMATIC DISEASES

BY

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The rise in plasma corticosteroid concentration after the administration of corticotrophin (ACTH) is an index of the functional reserve of the adrenal cortex (Eik-Nes, Sandberg, Nelson, Tyler, and Samuels, 1954).

Synacthen (β^{1-24} Ciba) consists of amino-acids 1 to 24 of the 39 amino-acid chain of natural ACTH and can be prepared in a crystalline form (Kappeler and Schwyzer, 1961), which is highly soluble in water and rapidly absorbed after intramuscular administration; it has activity equivalent to that of natural ACTH but its duration of action is shorter (Landon, James, Cryer, Wynn, and Frankland, 1964; Wood, Frankland, James, and Landon, 1965). Studies in patients with rheumatoid arthritis have shown that the simple Synacthen test is a satisfactory procedure for distinguishing patients with rheumatoid arthritis who have definite adrenal hypofunction produced by treatment with corticosteroid drugs from patients who have normal adrenal reserve (Greig, Browning, Boyle, and Maxwell, 1966). The test has obvious advantages over the ACTH infusion test, as it can be performed on out-patients over a half-hour period. Criteria for a normal response to the Synacthen test have previously been based on a knowledge of the plasma cortisol before injection of Synacthen, and both the increment in and the final level of plasma cortisol 30 minutes after injection (Greig and others, 1966). These criteria have been derived from observations made between 8 and 10 a.m.

In most instances, however, it is likely that the Synacthen test would be performed on out-patients somewhat later in the day, and the fact that plasma cortisol concentrations are higher in the morning than in the afternoon and evening (Bliss, Sandberg Nelson, and Eik-Nes, 1953) might conceivably distort the interpretation of results in out-patients at an afternoon clinic.

The present study was therefore undertaken to investigate the effect of the physiological diurnal rhythm of plasma cortisol on the results of the Synacthen test performed at different times of the day in a group of patients with rheumatic diseases.

Material and Methods

Eighteen subjects were studied, five males and thirteen females, whose ages ranged from 21 to 76 years (mean 51). Thirteen were hospital in-patients with mild rheumatoid arthritis, one had gout, one osteo-arthritis, and one erythema nodosum of unknown aetiology, and two were healthy subjects. In none of the patients was there clinical or laboratory evidence of hepatic, renal, or endocrine disorder and none had received corticosteroid drugs. The eighteen subjects were randomly divided into six groups of three (A to F in Table I).

TABLE I
ORDER OF PERFORMANCE OF SYNACTHEN TESTS
Eighteen patients divided into six groups of three (A-F) were subjected to three test procedures at different times of the day.

Group	Morning (8-9 a.m.)	Afternoon (2-3 p.m.)	Evening (8-9 p.m.)
A	1	2	3
B	1	3	2
C	2	1	3
D	3	1	2
E	2	3	1
F	3	2	1

None of the patients studied had received corticosteroid drugs, and all were in bed resting. A venous blood sample was taken, 250 μ g. Synacthen dissolved in 0.5 ml. sterile water was given by intramuscular injection, and 30 minutes later blood was removed. Plasma "cortisol" concentration was estimated in both samples by the rapid fluorimetric method of Mattingly (1962). Each individual was subjected to the test procedure on three occasions within a maximum period of 36 hours. These adrenal stimulation procedures were designated Test 1, 2, and 3 respectively. Table I shows the time sequence followed in the performance of the tests.

For instance the three patients in Group C were first

subjected to adrenal stimulation between 2 and 3 p.m. and the test was next repeated between 8 and 9 a.m. on the following day, the final test being performed between 8 and 9 p.m. in the evening. By these arrangements all the possible sequences for the adrenal stimulation tests were represented, thus compensating for any possible priming effect on the adrenal cortex produced by repeated stimulation.

Results

Table II shows the plasma cortisol responses to the Synacthen tests in each of the eighteen subjects who were studied at different times of the day. In each subject before the administration of Synacthen the plasma cortisol concentration was higher in the morning than in the evening, and in all but four (Nos. 5, 7, 11, and 12) the morning level was also

higher than the afternoon level. After Synacthen administration there was a rise in plasma cortisol in all subjects.

In the morning the mean and standard deviation of the plasma cortisol was 14.8 ± 3.2 $\mu\text{g./100 ml.}$; after Synacthen it rose to 30.1 ± 5.6 $\mu\text{g./100 ml.}$

In the afternoon the mean plasma cortisol rose from 11.9 ± 3.4 to 30.2 ± 7.2 $\mu\text{g./100 ml.}$, and in the evening it rose from 7.9 ± 2.5 to 29.0 ± 7.1 $\mu\text{g./100 ml.}$

These results are illustrated in the Figure.

Essentially therefore there was no difference in the levels of plasma cortisol reached 30 minutes after administration of Synacthen. Statistical analysis of the individual values at these times shows that a true

TABLE II
PLASMA CORTISOL CONCENTRATIONS ($\mu\text{g./100 ml.}$) BEFORE (BASAL) AND AFTER SYNACTHEN ADMINISTRATION (STIMULATED)
Three tests in each of eighteen subjects

Patient No.	Morning			Afternoon			Evening		
	Basal	Stimulated	Increment	Basal	Stimulated	Increment	Basal	Stimulated	Increment
1	13.6	25.2	11.6	10.0	28.5	18.5	10.0	27.0	17.0
2	18.9	33.5	14.6	13.0	30.0	17.0	8.0	24.5	16.5
3	15.0	28.2	13.2	10.3	28.5	18.2	8.5	28.0	19.5
4	9.2	26.7	17.5	8.5	28.5	20.5	3.5	21.5	18.0
5	11.9	26.7	14.8	12.8	32.3	20.3	10.4	29.7	19.3
6	16.3	24.5	8.2	10.5	27.8	17.2	5.7	25.5	19.8
7	14.0	26.0	12.0	17.0	28.5	12.5	11.4	28.5	17.1
8	18.0	36.5	18.5	9.5	29.5	20.0	7.3	38.5	31.2
9	13.0	33.8	20.8	11.6	28.5	16.9	5.9	32.6	26.7
10	16.0	22.0	6.0	6.5	14.5	8.0	6.5	15.0	8.5
11	12.0	39.0	27.0	15.5	38.5	23.0	9.0	33.5	24.5
12	11.9	29.6	17.7	18.3	36.0	17.7	8.9	29.2	20.3
13	19.3	23.8	4.5	14.8	23.3	8.5	5.0	19.5	14.5
14	16.3	30.2	13.9	7.0	22.5	15.5	7.2	30.7	23.5
15	17.3	29.5	12.2	12.6	44.9	32.3	6.7	38.6	31.9
16	11.2	34.9	23.7	10.2	29.9	19.7	8.8	24.5	15.7
17	11.6	28.2	16.6	9.3	27.8	18.5	5.0	30.5	25.5
18	20.4	42.7	22.3	16.0	43.5	27.5	13.5	44.0	30.5
Mean	14.8	30.1	14.2	11.9	30.2	18.4	7.9	29.0	21.1
Standard error of the Mean	± 0.75	± 1.32	± 1.10	± 0.80	± 1.70	± 1.37	± 0.64	± 1.67	± 1.49

Fig.—Mean plasma cortisol concentrations ($\mu\text{g./100 ml.}$) and two standard deviations before and after Synacthen test, repeated on three occasions in eighteen subjects. The basal cortisol levels fall during the day but the levels after Synacthen are the same throughout the day.

difference of 5.0 μg . cortisol per 100 ml. between the means obtained at each time of day, had such existed, could have been detected at the 5 per cent. level of significance.

The difference between the mean resting plasma cortisol values in the morning and evening is significantly different, the mean value in the morning being almost twice that in the evening.

Table II also shows the values for the increment in plasma cortisol attained 30 minutes after the injection of Synacthen in each individual patient. In the morning the mean and standard deviation of these values was $14.2 \pm 4.7 \mu\text{g}/100 \text{ ml.}$, which is significantly different from the afternoon and evening values of 18.4 ± 5.8 and $21.1 \pm 6.3 \mu\text{g}/100 \text{ ml.}$ ($P < 0.05$ and < 0.001) respectively.

Discussion

The varying of the sequence of repeat testing of the adrenal response to Synacthen shown in Table I, together with the transient action of intramuscular Synacthen, avoided the possibility that the plasma cortisol increments might have been influenced by a priming effect of multiple and sequential adrenocortical stimulations.

The results show that in each subject the basal plasma cortisol levels (before Synacthen administration) fell during the day-time, indicating normal diurnal rhythm. The plasma cortisol levels after Synacthen stimulation were, however, the same throughout the day. Thus, although the levels before Synacthen were higher in the morning than in the afternoon and evening, the capacity of the adrenal gland to secrete cortisol in response to Synacthen stimulation, as defined by maximum plasma cortisol levels, was the same throughout the day. This evidence indicates that two of the criteria of normal adrenal function derived from studies carried out between 8 and 10 a.m. are not valid

when patients are studied later in the day; since both the level of resting plasma cortisol and the increment in plasma cortisol after Synacthen exhibit a physiological diurnal change. Only the third criterion (*i.e.* the plasma cortisol level attained 30 minutes after Synacthen injection) has been found by us to remain unchanged throughout the day. It therefore seems reasonable to suggest that, when outpatient studies with Synacthen are performed, only this one criterion should be used.

Summary

(1) The response of the adrenal cortex to stimulation with the short-acting synthetic tetra-icosapeptide Synacthen (β^{1-24} Ciba) has been measured in the morning, afternoon, and evening in eighteen subjects with rheumatic disorders.

(2) The mean plasma cortisol levels after Synacthen stimulation were the same at each time of day. In view of the physiological diurnal variation of basal (unstimulated) plasma cortisol, it follows that the increment in plasma cortisol after Synacthen stimulation shows a diurnal variation, a larger rise being observed in the afternoon and evening than in the morning.

(3) These results suggest that, because of the diurnal rhythm of the basal cortisol level and the incremental response to Synacthen, both these criteria of adrenal function are unsuitable when outpatients are tested at different times of the day. The level of plasma cortisol attained 30 minutes after Synacthen administration would seem to be an acceptable criterion of the normal response, as this parameter remains constant throughout the day.

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Réponse du cortisol plasmatique au Synacthen (β^{1-24} Ciba) à différents moments de la journée chez des sujets atteints d'affections rhumatismales

RÉSUMÉ

(1) La réponse du cortex surrénal à la stimulation par le Synacthen (β^{1-24} Ciba), tétra-icosapeptide synthétique à action rapide, a été mesurée le matin, l'après-midi et le soir chez 18 sujets atteints d'affections rhumatismales.

(2) Le taux moyen du cortisol plasmatique après stimulation par le Synacthen fut le même à chaque moment de la journée. Etant données les variations journalières physiologiques du taux de base (en l'absence de stimulation) du cortisol plasmatique, on peut conclure que l'augmentation du cortisol plasmatique produit par la stimulation au Synacthen n'est pas constante dans la journée, l'augmentation observée l'après-midi et le soir étant plus importante que celle observée le matin.

(3) Ces résultats suggèrent que, en raison du rythme journalier du taux de base du cortisol et de la réponse à type d'accroissement au Synacthen, ces deux critères de la fonction surrénale ne sont pas valables chez des sujets non hospitalisés chez qui les tests sont faits à des moments différents de la journée. Le niveau du cortisol plasmatique atteint 30 minutes après l'administration de Synacthen semblerait être un critère acceptable de réponse normale, car ce paramètre reste constant au cours de la journée.

Respuesta del cortisol plasmático al Synacthen (β^{1-24} Ciba) en diferentes momentos del día en sujetos con enfermedades reumáticas

SUMARIO

(1) La respuesta de la corteza suprarrenal a la estimulación con Synacthen (β^{1-24} Ciba), un tetra icosapeptido sintético de acción corta, fué medida por la mañana, por la tarde y por la noche, en 18 sujetos con afecciones reumáticas.

(2) Las cifras medias del cortisol plasmático después de la estimulación con Synacthen fueron iguales en cada momento del día. En vista de las variaciones fisiológicas diurnas del cortisol plasmático basal (sin estimulación), se deduce que el incremento del cortisol plasmático producido por la estimulación con Synacthen no es constante durante el día, siendo el aumento por la tarde y por la noche superior al matutino.

(3) Estos resultados sugieren que, debido al ritmo diurno del nivel del cortisol basal y de la respuesta incrementadora con Synacthen, ambos criterios de la función suprarrenal no son válidos en sujetos sin hospitalizar, investigados a diferentes horas del día. El nivel del cortisol plasmático alcanzado 30 minutos después de la administración de Synacthen parece ser un criterio aceptable de respuesta normal, ya que este parametro permanece constante durante el día.