

First Department of Internal Medicine, University of Athens School of Medicine
(Director: Prof. E. A. Phocas), "King Paul" Hospital, Saint Thomas Str., Goudi,
Athens, Greece.

A SYNTHETIC POLYPEPTIDE—

β^{1-24} CORTICOTROPIN—IN THE DEPOT FORM

1. Adrenocorticotropic and Pituitary Responsiveness following Long-Term Intermittent Administration

By

S. A. BENOS, A. KOUTSOUKOS, H. BRAOUZI, A. GEORGOPOULOS
& F. CH. PAVLATOS

INTRODUCTION

In the past few years natural corticotropin has been extensively used in clinical practice for both diagnostic and therapeutic purposes. However, its administration has been limited on account of allergic reactions due to protein contamination and/or to antibody formation (2, 3, 4, 7, 11, 17, 20). It has been shown that synthetic peptides possess full steroidogenic and definitely less immunological activity (5, 8, 18). β^{1-24} corticotropin (Synacthen,* Ciba), a polypeptide synthesized by Kappeler & Schwyzer (9), has also been shown to have full adrenocorticotropic activity similar to that of natural corticotropin of 39 amino acids (18). The uses of Synacthen have been confined mainly to diagnostic purposes (21) because of its short duration of action. A long acting preparation, formulated on the basis of adsorption with an inorganic zinc complex (Depot-Synacthen,* Ciba) might be expected to possess a sustained adrenocorticotropic effect and therefore to be useful in clinical practice.

In this paper the adrenocorticotropic activity of Depot-

* Registered brand name.

Synacthen before and during its long-term intermittent administration, in forty asthmatic patients is reported. The pituitary responsiveness in a group of patients under the above regimen was also investigated. The clinical efficacy of Depot-Synacthen in bronchial asthma was studied in fifty patients, including the group of this paper, and the results are reported in a companion paper.

MATERIAL AND METHODS

Depot- β^{1-24} Corticotropin (Depot-Synacthen, Ciba, 42'915-Ba)*

This synthetic depot β^{1-24} corticotropin is a long-acting preparation formulated on the basis of adsorption with an inorganic zinc complex. When given intramuscularly 1 mg of this long-acting tetracosapeptide has been found to be approximately equivalent to 80 units of natural corticotropin-gel (1). In the present study the compound under investigation was given intramuscularly in the dose of 1.0 mg in the morning between 8 and 10 a.m.

Patients

Forty patients with severe chronic bronchial asthma, 22 females and 18 males, ranging in age from 14 to 73 years old were studied. Twenty-five of these patients had previously taken various amounts of corticosteroids for the symptomatic control of their asthma, as shown in Table 1. All the patients studied were hospitalized for the most part of their investigation, except a few under close follow-up as out-patients. Depot-Synacthen therapy was applied while the patients were under exacerbation of their asthma or in status asthmaticus. Depot-Synacthen was given once daily until satisfactory clinical improvement was achieved. Intermittent administration was thereafter established, the intervals between the injections depending upon the presenting symptoms.

Adrenocorticotrophic Studies

Before establishing the intermittent therapy with Depot-Synacthen its adrenocorticotrophic effect was evaluated in each of the 40 patients by determining the plasma 17-hydroxycorticosteroids (17-OHCS) levels before and after 6 hours of the intramuscular injection of 1.0 mg of the compound. The evaluation was extended in some of the above patients after 12 and 24 hours of the administration as well.

Hypothalamus-Pituitary-Adrenal Axis Study

The assessment of the function of the hypothalamus-pituitary-adrenal axis after long-term administration of Depot-Synacthen was done in 14 of the above

* Registered brand name.

TABLE 1

Values of 17-OHCS in Plasma of 40 Asthmatic Patients before and after
1 mg Depot-Synacthen.

No.	Patient age, sex	Duration of asthma	Prednisone dose, duration*	Plasma 17-OHCS (μ g/100 ml)			
				Base- line	After 1 mg depot-synacthen		
					6 hours	12 hours	24 hours
1	A.S., 60, F	25 yrs	15 mg-10 yrs	12.2	30.0		
2	I.V., 33, F	9 yrs	15 mg-10 mo.	10.0	23.4		
3	E.K., 42, F	17 yrs		19.1	25.0		
4	G.S., 39, F	11 yrs	15 mg- 8 yrs	17.3	47.8	44.0	20.2
5	A.D., 30, F	15 yrs	10 mg- 7 yrs	8.0	26.0		
6	T.M., 31, F	1 yr	10 mg- 1 yr	20.0	37.1		
7	A.V., 37, F	10 yrs	10 mg- 7 yrs	5.0	24.4	15.2	6.3
8	E.K., 70, F	12 yrs	10 mg- 5 yrs	24.4	38.0	41.1	
9	E.V., 44, F	9 yrs	15 mg- 7 yrs	20.0	31.3	35.0	15.1
10	A.E., 40, F	1.5 yrs	15 mg- 6 mo.	22.1	58.0		
11	K.L., 35, F	3 yrs	20 mg- 1 yr	25.0	44.1		
12	K.K., 52, F	12 yrs		11.2	39.0		
13	M.P., 73, F	15 yrs		17.8	70.0		
14	M.M., 38, F	1 yr		19.0	42.9		
15	K.M., 50, F	1.5 yrs	15 mg- 1 yr	18.9	60.1		
16	C.S., 49, F	1 yr		14.0	46.8		
17	M.T., 38, F	12 yrs		13.8	49.0		
18	G.S., 39, F	12 yrs	15 mg- 8 yrs	14.7	47.9		
19	A.M., 35, F	2 yrs		23.0	45.0		
20	V.A., 73, F	4 yrs		20.8	42.2		
21	E.K., 33, F	5 yrs	10 mg- 2 yrs	8.0	35.0		
22	E.D., 28, F	3 yrs		18.8	45.3		
23	A.M., 50, M	4 yrs	10 mg- 2 yrs	12.2	23.0		
24	I.A., 60, M	4 yrs	12½ mg-1 yr	8.0	22.1	20.3	10.0
25	G.B., 51, M	4 yrs		16.3	29.0	27.1	26.8
26	A.K., 40, M	3 yrs		15.0	26.2	31.0	26.9
27	A.K., 62, M	5 yrs	12½ mg-3 yrs	16.1	35.0	23.2	3.9
28	N.Z., 47, M	1 yr		3.0	30.9	40.0	10.0
29	E.V., 50, M	8 yrs	15 mg-1.5 yrs	23.8	54.0		22.2
30	G.G., 45, M	4 mo.	15 mg-4 mo.	20.0	40.0		28.1
31	K.K., 67, M	30 yrs		12.0	27.1	38.8	32.0
32	D.K., 18, M	2 yrs	10 mg-1 yr	5.0	30.1	25.0	12.2
33	V.Z., 51, M	4 yrs	10 mg-2 yrs	5.0	28.9		
34	G.M., 50, M	7 mo.	15 mg-3 mo.	7.1	28.0		

TABLE 1 (cont.)

No.	Patient age, sex	Duration of asthma	Prednisone dose, duration*	Plasma 17-OHCS ($\mu\text{g}/100\text{ ml}$)			
				Base- line	After 1 mg depot-synacthen		
					6 hours	12 hours	24 hours
35	A.P., 34, M	6 yrs	15 mg-6 yrs	14.0	35.0		
36	A.G., 43, M	5 yrs	10 mg-2 yrs	20.1	39.2		
37	G.D., 45, M	10 mo.		21.0	38.1		
38	I.L., 39, M	20 yrs	10 mg-5 yrs	19.0	37.3		
39	N.L., 14, M	7 yrs	10 mg-2 yrs	8.0	25.2		
40	P.K., 54, M	24 yrs		16.5	54.0	38.2	18.0
Mean				15.13	37.7	31.5	17.8
$\pm$ SE				± 0.94	± 1.85	± 2.77	± 2.52

* Corticosteroids were taken occasionally for symptomatic relief.

patients by evaluating the 6-hour responsiveness of the adrenals as described above and in 10 patients the pituitary responsiveness by applying the Methopyrapone test.

Plasma 17-Hydroxycorticosteroids (17-OHCS)

Plasma 17-OHCS levels were determined by the method of Silber & Porter (19) as modified by Peterson *et al.* (12, 13). This method measures both protein-bound and non-protein-bound plasma cortisol, as well as cortisone, tetrahydrocortisone and tetrahydrocortisol (in small amounts), but non glucuronides, which constitute about half of the total plasma corticosteroids and their metabolites in the presence of normal levels of transcortin. Peterson (12) found a mean normal value of $15 \pm 5.5 \mu\text{g}/100\text{ ml}$ in samples taken at 8 a.m.

Urinary 17-OHCS

Urinary 17-OHCS were determined by a modification of the method of Reddy (15) using the colorimetric method of Silber & Porter (19) (normal values 4-10 mg/24 hours).

Methopyrapone Test (Metyrapone, Metopirone,* SU-4885 Test)

The intravenous methopyrapone test was used, as described by Gold, DiRaimondo & Forsham (6). According to this method the Methopyrapone is infused as the ditartrate salt in a dose of 30 mg per kg body weight in 500 ml saline solution over a four-hour period. On that day and the previous day a 24-hour

* Registered brand name.

ACTH 1-24 Zn 1 MG

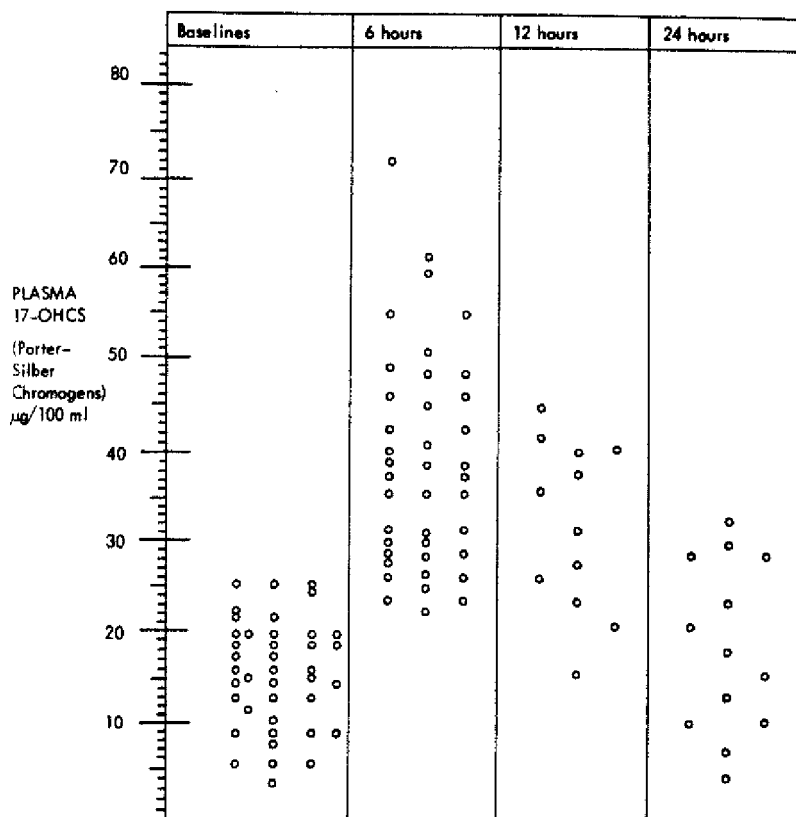


Fig. 1.

Levels of 17-OHCS at 0, 6, 12, and 24 hours in plasma specimens from asthmatic patients who were given a single injection of 1 mg Depot-Synacthen intramuscularly.

urine collection was made for determination of 17-OHCS as described above. A normal response is characterized by the doubling, at least, of the urinary 17-OHCS.

Clinical Studies

The clinical efficacy of depot-Synacthen was studied in 50 asthmatic patients including the group of this paper. The therapeutic results as well as the extra-therapeutic effects are reported in detail in a companion paper.

($\mu\text{g}/100\text{ml}$) Mean plasma 17-OHCS

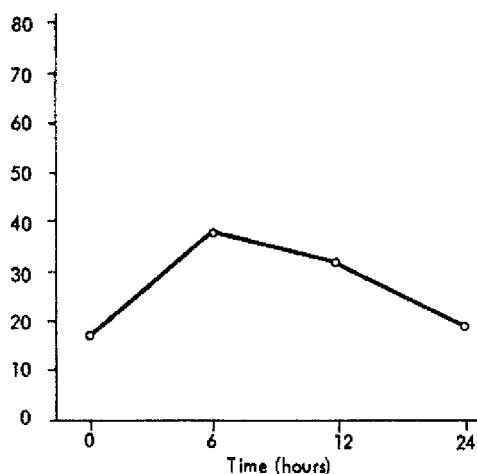


Fig. 2.

Mean plasma 17-OHCS concentration in 40 asthmatic patients after 1 mg Depot-Synacthen.

RESULTS

The age and sex of the patients as well as the duration of their asthma and data concerning previous treatment with corticosteroids are presented in Table 1.

Adrenocorticotropic Studies

The baseline studies in forty asthmatic patients before the initiation of treatment with Depot-Synacthen are shown in Table 1 and depicted in Fig. 1 and Fig. 2.

The individual values of plasma 17-OHCS 6, 12, and 24 hours before and after a single intramuscular injection in the morning of 1 mg Depot-Synacthen are as follows:

Basal plasma 17-OHCS concentration ranged from 3.0 to 25.0 with a mean value and standard error (S.E.) of $15.13 \pm 0.94 \mu\text{g}/100\text{ml}$. At 6 hours all patients showed a notable rise of plasma 17-OHCS to levels between 22.1 and 70.0 with a

TABLE 2

Levels of Plasma 17-OHCS and the per cent Increase Above the Baseline Values before and after a Definite Period of Treatment.

Therapy with depot- β^{1-24} corticotropin (Depot-Synacthen®)									
No. of pt	Plasma 17-OHCS		% increase	Treatment given			Plasma 17-OHCS ($\mu\text{g}/100\text{ ml}$)		% increase
	Before treatment			Initial No. of daily in-jections	Intermittent Intervals between in-jections (days)	Duration of treatment	After treatment		
	Base-line	6 hours					Base-line	6 hours	
2	10.0	23.4	134.0	1 $\times$ 6	4-5	4 mo.	5.5	41.2	649.0
3	19.1	25.0	30.8	1 $\times$ 29	2-15	4 mo.	8.5	34.0	300.0
4	17.3	47.8	176.3	1 $\times$ 8	5-6	1 mo.	19.2	40.4	110.4
						4 mo.	15.0	47.9	219.3
5	8.0	26.0	225.0	1 $\times$ 15	2-4	1.5 mo.	5.1	25.7	403.9
6	20.0	37.1	85.5	1 $\times$ 11	2-5	4 mo.	24.9	46.4	86.3
7	5.0	24.4	388.0	1 $\times$ 5	3-4	2 mo.	5.2	31.3	501.9
8	24.4	38.0	55.7	1 $\times$ 25	-	25 days	13.7	42.1	207.2
9	20.0	31.3	56.5	1 $\times$ 7	2-3	1 mo.	13.9	40.4	190.6
						2 mo.	7.0	23.5	235.7
23	12.2	23.0	88.3	1 $\times$ 7	4-5	3.5 mo.	2.1	9.3	342.8
24	8.0	22.1	176.2	1 $\times$ 7	5-6	1 mo.	8.8	20.0	127.2
						2 mo.	14.5	42.6	193.7
						4 mo.	9.5	30.2	217.8
26	15.0	26.2	74.6	1 $\times$ 4	15-20	1.5 mo.	20.0	40.0	100.0
						3 mo.	17.2	50.7	194.7
29	23.8	54.0	126.8	1 $\times$ 4	8-10	1 mo.	15.2	62.0	307.8
						3 mo.	10.1	37.2	268.3
30	20.0	40.0	100.0	1 $\times$ 3	2-3	1 mo.	19.8	44.1	122.7
33	5.0	28.9	478.0	1 $\times$ 4	2-3	3 mo.	8.5	45.5	435.2

mean and S.E. $37.7 \pm 1.85 \mu\text{g}/100\text{ ml}$. This increase from 15.13 to $37.7 \mu\text{g}/100\text{ ml}$ represents an average increment of 149.17 per cent above the baseline value. The response at 12 hours in 12 patients ranged from 15.2 to 44.0 with a mean value and S.E. of $31.5 \pm 2.77 \mu\text{g}/100\text{ ml}$ of plasma 17-OHCS, which represents an increase equivalent to 108.19 per cent above the baseline value. The plasma 17-OHCS concen-

($\mu\text{g}/100\text{ ml}$) Plasma 17-OHCS

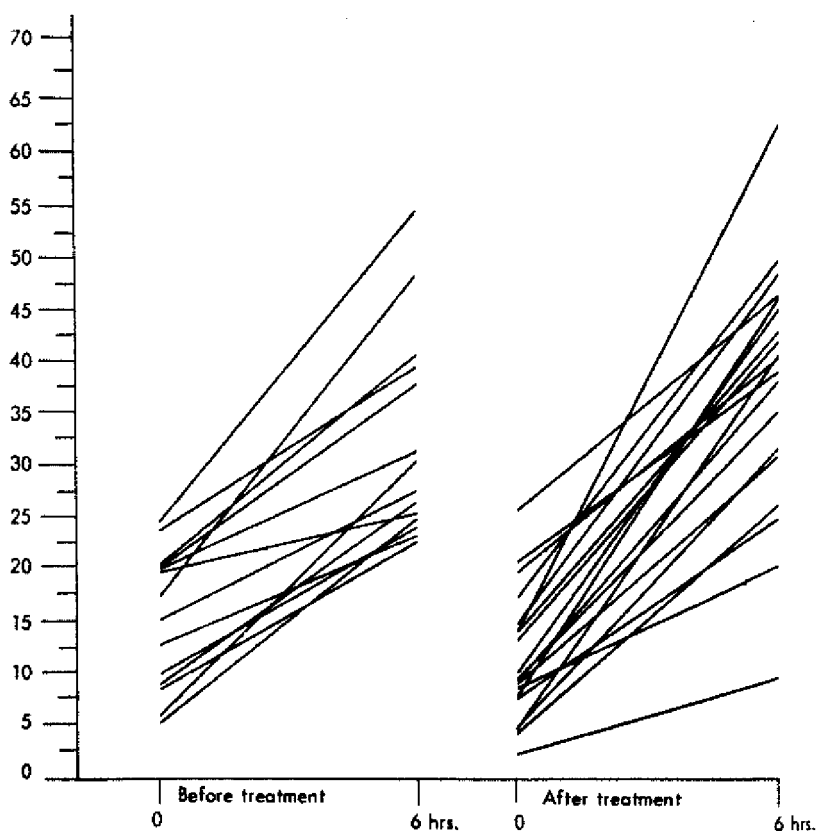


Fig. 3.

Responsiveness of the adrenal cortex after long-term administration of Depot-Synacthen. Individual levels of plasma 17-OHCS before and after 6 hours of an intramuscular injection of 1 mg Depot-Synacthen (6-hour intramuscular Depot-Synacthen test).

tration after 24 hours was in 13 patients between 3.9 and 32.0 with a mean and S.E. of $17.8 \pm 2.52 \mu\text{g}/100\text{ ml}$, only slightly above (17.64 per cent) the baseline levels.

Fig. 2 shows the pattern of plasma 17-OHCS response to a single intramuscular injection of Depot-Synacthen (mean values).

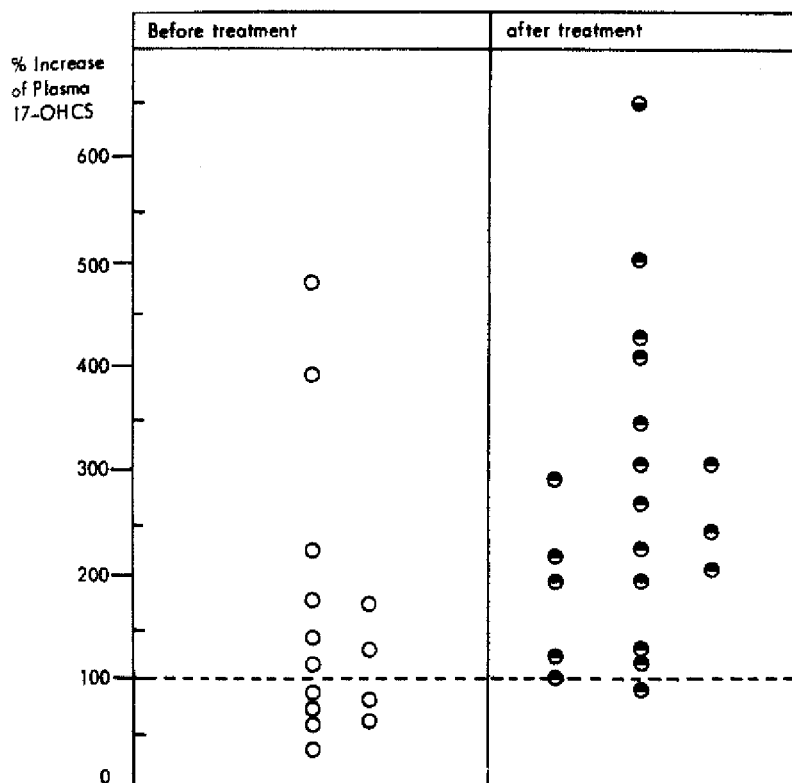


Fig. 4.

Six-hour intramuscular Depot-Synacthen test. Responsiveness of the adrenal cortex before and after long-term administration of Depot-Synacthen. Per cent increase of plasma 17-OHCS above the baseline values after 6 hours of an intramuscular injection of 1 mg Depot-Synacthen.

Hypothalamus-Pituitary-Adrenal Axis Study

Six-hour responsiveness after long-term administration of Depot-Synacthen. The responsiveness of the adrenal cortex 6 hours after an intramuscular injection of 1 mg Depot-Synacthen in 14 patients is shown in Table 2 and depicted in Fig. 3. In Table 2 the baseline studies, the number of daily injections given initially, the intervals between the injections and the duration of intermittent therapy are also set out. The

percent increase above the baseline values of plasma 17-OHCS (six-hour responsiveness) both before the initiation and after a definite period of intermittent therapy was also calculated. The baseline values of plasma 17-OHCS after 25 days to 4 months intermittent therapy with Depot-Synacthen fluctuated between 2.1 and 24.9 $\mu\text{g}/100\text{ ml}$. After the intramuscular injection of 1 mg Depot-Synacthen the levels of 17-OHCS in plasma ranged from 9.3 to 62.0 $\mu\text{g}/100\text{ ml}$. The corresponding percent increase fluctuated between 86.3 and 649.0, as compared to the per cent increase in the baseline studies, before initiation of the Depot-Synacthen therapy, which ranged from 30.8 to 478.0.

All but one the 20 individual responses obtained after long term intermittent administration of Depot-Synacthen were above 100 per cent, whereas only 7 of 14 responses in the

TABLE 3
Hypothalamus-Pituitary-Adrenal responsiveness. Values of Urinary 17-OHCS before and the Day of Methopyrapone Test, as Described in the Text.

Therapy with Depot- β^{1-24} corticotropin (Depot-Synacthen®)						
No. of pt	Treatment given			After treatment: Methopyrapone test		
	Initial No. of daily injections	Inter-vals between injections (days)	Dura-tion of treat-ment	Base-line	Day of test	% in-crease
2	1 $\times$ 6	4-5	11 mo.	3.0	14.0	366
4	1 $\times$ 8	5-6	8 mo.	6.0	10.5	75
10	1 $\times$ 5	5-8	12 mo.	5.3	7.6	43
15	1 $\times$ 2	2	2 mo.	4.5	12.0	166
24	1 $\times$ 7	5-6	9 mo.	5.2	13.0	150
27	1 $\times$ 2	5-7	6.5 mo.	7.1	11.2	57
29	1 $\times$ 4	8-10	9 mo.	9.8	19.8	102
34	1 $\times$ 3	3-4	3 mo.	5.7	13.5	137
36	1 $\times$ 1	6-7	4 mo.	5.5	14.9	171
39	1 $\times$ 2	4-5	9 mo.	5.9	11.8	100

baseline studies before treatment reached levels above 100 per cent (Table 2 and Fig. 4).

Methopirapone Test

The response to intravenous Methopirapone test in 10 patients after long-term intermittent administration of Depot-Synacthen for 2 to 12 months are presented in Table 3. The basal levels of urinary 17-OHCS ranged from 3.0 to 9.8 mg/24 hours. On the day of the test the levels of 17-OHCS in the urine were between 7.6 and 19.8 mg/24 hours. All the individual values were increased above the control levels, with a per cent increment ranging between 43 and 366.

DISCUSSION

The adrenocorticotrophic activity of the depot- β^{1-24} corticotropin (Depot-Synacthen) in our patients was found to be satisfactory, maintaining continuously elevated plasma cortisol levels for 24 hours. The plasma 17-OHCS levels at 6 and 12 hours were quite high probably more than the one needed to achieve an adequate therapeutic response. Possibly smaller doses might prove to possess the same therapeutic efficacy minimizing, on the other hand, the slight extra-therapeutic effects of Depot-Synacthen (see companion paper).

The responsiveness of the adrenal cortex after long-term intermittent administration of Depot-Synacthen, as assessed by the six-hours plasma cortisol response to 1 mg of this compound (6-hour intramuscular Depot-Synacthen test), was definitely increased, as compared with the baseline values before the initiation of the treatment. This has been found with natural ACTH by *Reed et al.* (16) and by *Killborn et al.* (10).

The hypothalamus-pituitary-adrenal responsiveness following long-term intermittent administration, as assessed by the intravenous Methopirapone test, was satisfactory, except one patient (No. 10) in whom little response was demonstrated. Administration of natural corticotropin has been reported by *Plager & Cushman* (14) and *Reed et al.* (16) to produce a

diminished endogenous corticotropin response to Methopyrone. Whether the more or less satisfactory responses in our patients might be attributed to the synthetic β^{1-24} corticotropin or to the intermittent administration of this compound needs further study.

SUMMARY

The adrenocorticotrophic activity of Depot-Synacthen was studied in 40 patients by measuring plasma 17-OHCS at 0, 6, 12 and 24 hours after an intramuscular injection of 1 mg of the compound. The adrenal and pituitary responsiveness after long-term intermittent administration of Depot-Synacthen was investigated in a group of the above patients, by applying the 6-hour intramuscular Depot-Synacthen test and the intravenous Methopyrone test respectively.

The baseline studies showed an adrenocorticotrophic action extending through 24 hours, with a peak at 6 hours after the injection. All but one of the patients studied showed a definitely increased adrenal responsiveness to Depot-Synacthen after its long-term administration. The response to the intravenous Methopyrone test was satisfactory except in one patient.

ACKNOWLEDGEMENT

This work was supported by a grant from Ciba Research Laboratories, Switzerland, which also kindly provided the depot- β^{1-24} corticotropin (Depot-Synacthen®; Ciba).

REFERENCES

1. *Besser, G. M., Butler, P. W. P. & Plumpton, F. S.*: Adrenocorticotrophic action of long-acting tetracosactrin compared with corticotrophin-gel. *Brit. med. J.*, 4, 391, 1967.
2. *Charpin, J., Zafiropoulos, A., Aubert, J., Ohresser, Ph. & Boutin, Ch.*: Données actuelles concernant l'allergie à l'ACTH. *La Presse Medicale*, 72, 3025, 1964.
3. *Eisalo, A., Leskinen, O. & Oka, M.*: Fatal anaphylactic shock following corticotropin. *Acta Med. Scand.*, 155, 1, 1956.
4. *Ewans, H. M., Sparks, L. L. & Dixon, J. S.*: The physiology and chemistry of adrenocorticotropin. In: *Harris, C. W. & Donovan, B. T. (Eds.). The Pituitary Gland*. Vol. 1, p. 317. Butterworths, London, 1965.

5. *Felber, J. P., Ashcroft, S. H. J., Villanueva, A. & Vannotti, A.*: Antibodies to synthetic corticotropin. *Nature (London)*, 211, 654, 1966.
6. *Gold, E. M., DiRaimondo, V. C. & Forsham, P. H.*: Quantitation of pituitary corticotropin reserve in man by use of an adrenocortical 11-beta hydroxylase inhibitor (SU-4885). *Metabolism*, 9, 3, 1960.
7. *Hill, B. H. R. & Sawinburn, P. D.*: Death from corticotropin. *Lancet*, 1, 1218, 1954.
8. *Imura, H., Sparks, L. L., Grodsky, G. M. & Forsham, P. H.*: Immunologic studies of adrenocorticotrophic hormone (ACTH): Dissociation of biologic and immunologic activities. *J. Clin. Endocr.*, 25, 1361, 1965.
9. *Kappeler, H. & Schwyzler, R.*: Die Synthese eines Tetracosapeptides mit der Aminosäuresequenz eines hochactiven abbauproduktes des β -Corticotropins (ACTH) aus Schweinehypophysen. *Helv. Chim. Acta*, 44, 422, 1965.
10. *Kilborn, J. R. & Ronson, A. O.*: Effect of corticosteroid and corticotrophin therapy on adrenocortical function in bronchial asthma. *Thorax*, 20, 422, 1965.
11. *Landon, J., Friedman, M. & Greenwood, F. C.*: Antibodies to corticotrophin and their relation to adrenal function in children receiving corticotrophin therapy. *Lancet*, 1, 652, 1967.
12. *Peterson, R. E., Karrer, A. & Guerra, S. L.*: Evaluation of Silber-Porter procedure for determination of plasma hydrocortisone. *Analytical Chemistry*, 29, 144, 1957.
13. *Peterson, R. E., Wyngaarden, J. B., Guerra, S. L., Brodie, B. B. & Bunim, J. J.*: The physiological disposition and metabolic fate of hydrocortisone in man. *J. Clin. Invest.*, 34, 1779, 1955.
14. *Plager, J. E. & Cushman, P. Jr.*: Suppression of the pituitary-ACTH response in man by administration of ACTH or cortisol. *J. Clin. Endocr.*, 22, 147, 1962.
15. *Reddy, W. J.*: Modification of the Reddy-Jenkins-Thorn Method for the estimation of 17-hydroxycorticoids in urine. *Metabolism*, 3, 489, 1954.
16. *Reed, P. I., Clayman, Ch. B. & Palmer, W. L.*: Adrenocortical and pituitary responsiveness following long-term, high dosage corticotrophin administration. *Ann. Int. Med.*, 61, 1, 1964.
17. *Rosenblum, A. H. & Rosenblum, P.*: Anaphylactic reaction to adrenocorticotrophic hormone in children. *J. Pediat.*, 64, 387, 1964.
18. *Schuler, F. W., Schär, B. & Desaulles, P.*: Zur Pharmakologie eines ACTH-wirksamen, vollsynthetischen Polypeptids, des β^{1-24} corticotropins, CIBA 30920-Ba, Synacthen. *Schweiz. Med. Wschr.*, 93, 1027, 1963.
19. *Silber, R. H. & Porter, G. C.*: The determination of 17,21-dihydroxy-20-ketosteroids in urine and plasma. *J. Biol. Chem.*, 210, 923, 1954.
20. *Wilson, L. A.*: Proteinshock from intravenous ACTH. *Lancet*, 2, 478, 1951.
21. *Wood, J. B., Frankland, A. W., James, V. H. T. & Landon, J.*: A rapid test of adrenocortical function. *Lancet*, 1, 243, 1965.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.