

MEASUREMENT OF THE ADRENAL CORTEX RESERVE USING DEPOT β^{1-24} - CORTICOTROPHIN. I — IN ADRENAL INSUFFICIENCY

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SUMMARY

Adrenal reserve was assessed by measuring plasma cortisol 4 and 6 hours after intramuscular injection of 1 mg Synacthen Depot in 87 normal subjects (95 tests), in 13 patients with primary adrenal insufficiency (26 tests) and in 21 patients with secondary adrenal insufficiency (30 tests). With this Synacthen Depot test differentiation between normals (plasma cortisol 4-6 hours after Synacthen Depot = 62.9 ± 12.26 $\mu\text{g}/100$ mg) and adrenal insufficiency, either primary (9.8 ± 3.67 $\mu\text{g}/100$ ml) or secondary (21.3 ± 12.65 $\mu\text{g}/100$ ml) was possible in all cases (p less than 0.001). On the contrary in some patients with secondary hypoadrenalism a single injection of Synacthen Depot did not secure their differentiation from patients with primary hypoadrenalism and further injections were required to establish the diagnosis.

The levels of cortisol and of other metabolites in plasma and in urine may be low or normal in adrenal insufficiency, whether primary or secondary, therefore, the measurement of basal levels has limited diagnostic value. The method used universally to test adrenocortical function is that of demonstrating an absence of cortical response to stimulation by corticotrophin (ACTH). Stimulation by ACTH assesses the ability of the adrenal cortex to respond to the stimulus of its trophic hormone, thus establishing the size of reserves in the adrenal cortex. Information is thus obtained with regard to the potential of the adrenal cortex to respond to *stress*.

Since Thorn and his co-workers described a test of adrenal stimulation based upon changes in urinary 17-ketosteroids (17-KS) after an intravenous infusion of ACTH (Renold et al 1952; Thorn et al 1953), many other papers were published about the stimulation of the adrenal cortex on the diagnosis of adrenal insufficiency (Eik-Nes et al 1954; Christy et al 1956; Engbring et al 1956; Gold and Starr 1959; Maynard et al 1966; Musa and Dowling 1967).

It has been clear since the early studies of adrenal function that multiple stimulations with ACTH, over periods of 3 to 6 days, must be given in cases of adrenocortical atrophy due to hypopituitarism (Thorn et al 1953; Prunty 1956; Sandberg et al 1957). Numerous studies have been carried out concerning suprarenal reserves after steroid therapy (Christy et al 1956; Sandberg et al 1957; Farmer et al 1961; Shuster and Williams 1961; Treadwell et al 1963; Galvão-Teles et al 1971).

A more sophisticated test of adrenal function was described by Rose et al (1970).

Progress was achieved in the field of tests of adrenal function with the introduction of β^{1-24} - corticotrophin (Synacthen-Ciba), a synthetic preparation. This syn-

thetic ACTH consists of the partial sequence 1-24 of the ACTH molecule. The approved name of this substance is tetracosactrin. Numerous studies have shown that Synacthen has the same physiological and pharmacological properties as natural ACTH (Karl 1963; Grahame-Smith et al 1967).

This synthetic polypeptide can be determined by weight and biological assay is not necessary; it is devoid of foreign proteins.

Landon et al (1964) secured identical cortical stimulation in normal individuals and in patients with adrenal atrophy secondary to cortisone therapy, when they infused 100 $\mu\text{g}/\text{hour}$ Synacthen or 10 IU/hour of natural corticotrophin for six hours.

These studies led Wood et al (1965) to describe a test of adrenal stimulation, in which the response was assessed by determining the plasma cortisol (11-OHCS measured by the method of Mattingly, 1962) 30 minutes after an intramuscular injection of 250 μg Synacthen. Moncloa et al (1966) showed that stimulation at one hour was more evident.

Sustained action β^{1-24} ACTH (Synacthen Depot), introduced for therapeutic purposes, was formed by absorbing the active substance upon zinc phosphate (Besser et al 1967). It was found (El-Shaboury 1968) that 0.5 to 1 mg of sustained-action Synacthen administered intramuscularly was equivalent in potency to 40 IU of corticotrophin gel. Besser et al (1967) compared doses of Synacthen Depot of 1 and 2 mg with 80 IU of corticotrophin gel and they found no difference in plasma cortisol levels in the first four hours.

Galvão-Teles et al (1971) found that the maximum rise of plasma cortisol occurred 6 hours after an intramuscular injection of 1 mg Synacthen Depot, after which time the levels were maintained for some 10 hours. These levels began to fall at 16 hours but they were still significantly greater than the basal levels at 24 hours. No response was noticed in patients with Addison's disease and an intermediate response was displayed in secondary hypoadrenalism. A test of adrenal function was suggested, based upon the rise of plasma cortisol 4 to 6 hours after the administration of Synacthen Depot (Galvão-Teles et al 1971).

As Synacthen Depot contains no foreign protein and lacks the 25-39 moiety in its molecule, hypersensitivity reactions are much less common. No hypersensitivity reactions to Synacthen have been described during the use of the drug for diagnostic tests.

This paper is concerned with measurement of adrenal reserves in normal subjects and in patients with primary and secondary adrenal insufficiency, using the 4-6 hours Synacthen Depot test as described in a previous publication (Galvão-Teles et al 1971).

MATERIAL AND METHOD

Subjects studied

Normal—87 subjects (21 male and 66 female), aged 18 to 64 years, selected from among healthy doctors, nurses, medical students, other volunteers and persons examined at the hospital and found to be free from endocrine, hepatic or renal diseases.

—*Patients with primary adrenal insufficiency*—17 patients: 13 with Addison's disease and 4 with total bilateral adrenalectomy on corticosteroids (2 cases of Cushing's syndrome and 2 of carcinoma of the breast). Those patients who were receiving maintenance steroid therapy which interfered with the measurement of cortisol had that treatment replaced by dexamethasone during the test period.

Patients with secondary adrenal insufficiency—21 patients: 14 on steroids (5 to 50 mg of prednisone or its equivalent for a period of 1 month to 10 years); 6 with panhypopituitarism (1 idiopathic, 1 with the empty sella syndrome, 4 after total hypophysectomy for pituitary tumours); 1 with an isolated ACTH deficiency.

Protocol and Method

The dose of 1 mg β^{1-24} - corticotrophin depot (*Synacthen Depot* Ciba) was administered intramuscularly between 8 and 10 a.m. Blood was taken immediately before and at 4 and 6 hours after the injection. The tests were carried out on out-patients and the patients were not fasting or at rest.

Throughout the paper the name *Synacthen Depot* will be used to refer to the polypeptide.

The effect of *Synacthen Depot* was assessed by measuring the 11-hydroxycorticosteroids (11-OHCS) in the plasma (Mattingly's method, 1962). In this method, in addition to cortisol, small quantities of corticosterone and unknown fluorescent substances are also measured. In this paper the term *plasma cortisol* refers to total plasma 11-OHCS.

RESULTS

The 4-6 hours Synacthen depot test in normal subjects

The results of 95 tests of *Synacthen Depot* in 87 normal subjects are shown in fig. 1. The mean (\pm SD) of basal levels of plasma cortisol in 26 tests performed in 21

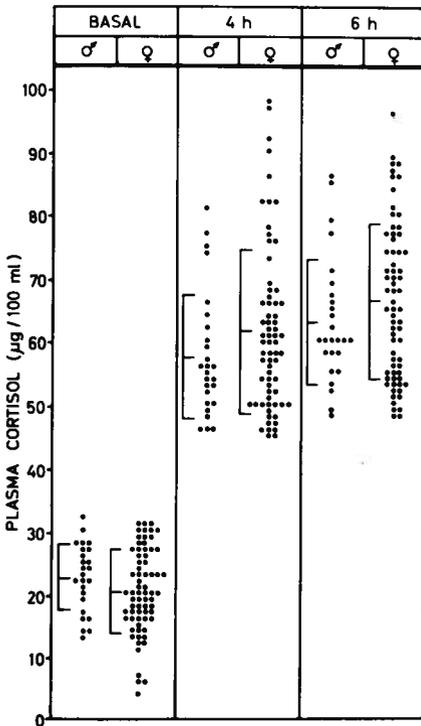


Fig. 1 — *Plasma cortisol in 87 normal female and male subjects, before and after Synacthen Depot. Means and SD*

male subjects was $22.5 \pm 5.32 \mu\text{g}/100 \text{ ml}$. Four and six hours after the injection of 1 mg Synacthen Depot the plasma cortisol levels were 57.7 ± 9.87 and $63.0 \pm 9.99 \mu\text{g}/100 \text{ ml}$ respectively. Results of 69 tests in 66 female subjects were identical to those of the male subjects (basal: $20.3 \pm 6.67 \mu\text{g}/\text{ml}$; 4 hours: $61.7 \pm 13.07 \mu\text{g}/100 \text{ ml}$; 6 hours: $66.3 \pm 12.30 \mu\text{g}/100 \text{ ml}$). In neither sex was there a significant difference between the plasma cortisol levels at 4 and at 6 hours, nor was age (18 to 64 years) a factor of variation.

Thus, the values in normal subjects of plasma cortisol can be considered together, as a single group, and the values at 4 and 6 hours (4-6 hours) can also be grouped together. In this way, the values for the normal group were as follows: basal: $20.9 \pm 6.39 \mu\text{g}/100 \text{ ml}$ (range 4-32); 4 to 6 hours: $62.9 \pm 12.26 \mu\text{g}/100 \text{ ml}$ (range: 45-98) (fig. 2) (p less than 0.001). The normal value in a Synacthen Depot test for plasma cortisol was taken to be equal to or greater than $45 \mu\text{g}/100 \text{ ml}$ in both samples of blood at 4 and at 6 hours after the injection, irrespective of the basal value.

The Synacthen Depot test was repeated in 6 normal subjects and was found to be reliable (table 1).

The Synacthen Depot test was compared with the 0.25 mg ordinary Synacthen test in 9 normal subjects. As can be seen in figure 3, the mean plasma cortisol after administration of Synacthen Depot was higher than that obtained after ordinary Synacthen and, in all cases, the level after Synacthen Depot was greater than after ordinary Synacthen.

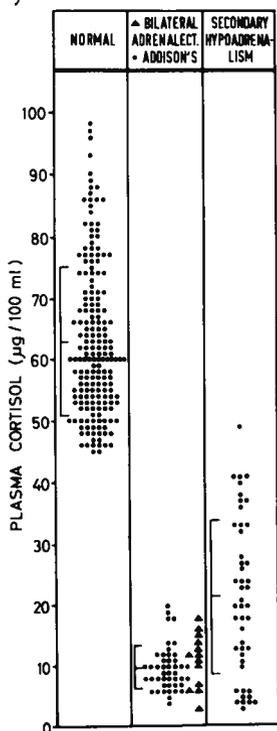


Fig. 2 — Plasma cortisol 4 to 6 hours after Synacthen Depot in normals, in primary and in secondary adrenal insufficiency: means \pm SD

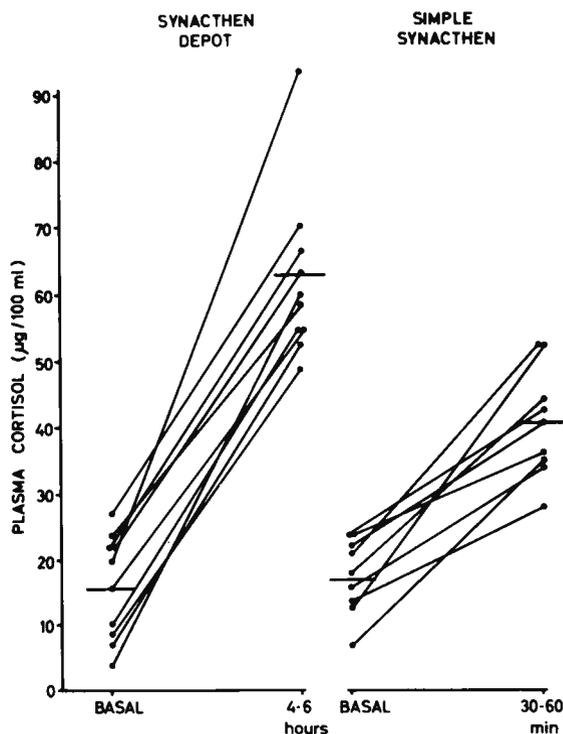


Fig. 3 — Comparison of the effects of Synacthen Depot and of Simple Synacthen upon plasma cortisol in 9 normal individuals

Table 1

Subject	Plasma cortisol ($\mu\text{g}/100\text{ ml}$)		
	Basal	4 H	6 H
1	22	46	60
	26	53	60
2	27	62	71
	16	66	79
3	32	56	60
	22	56	60
	16	46	48
4	20	49	58
	25	64	66
5	19	61	—
	17	60	62
6	25	50	54
	18	54	65
	20	50	59

The 4-6 hours Synacthen Depot test in the diagnosis of adrenal insufficiency

Figure 2 shows the values of basal plasma cortisol and the values at 4-6 hours after administration of Synacthen Depot to the following groups: normal, primary adrenal insufficiency, secondary adrenal insufficiency. There was no appreciable rise of plasma cortisol in the 26 tests carried out in 13 patients with Addison's disease. In this group the basal plasma cortisol (mean \pm SD: $8.8 \pm 4.51 \mu\text{g}/100\text{ ml}$) and the levels 4-6 hours after Synacthen Depot (mean \pm SD: $9.8 \pm 3.67 \mu\text{g}/100\text{ ml}$) were significantly different from the values obtained in normal subjects (p less than 0.001). Similar results were found in 7 tests carried out in 4 patients who had had bilateral adrenalectomy.

With this test, the differentiation between the normal group and the group with primary adrenal insufficiency was absolute and there was no overlap of values. Cortisol levels were below $20 \mu\text{g}/100\text{ ml}$ (range: 4-20 $\mu\text{g}/100\text{ ml}$), except in one case where the level was $20 \mu\text{g}/100\text{ ml}$.

Thirty Synacthen Depot tests were performed in 21 patients with secondary adrenal insufficiency. The basal plasma cortisol (mean \pm SD: $9.0 \pm 6.44 \mu\text{g}/100\text{ ml}$) and plasma cortisol 4 to 6 hours after the injection (mean \pm SD: $21.3 \pm 12.65 \mu\text{g}/100\text{ ml}$) were significantly different from the levels obtained in normal subjects (p less than 0.0001). In only one case did the plasma cortisol level after injection reach a normal level (range 3 to 52 $\mu\text{g}/100\text{ ml}$) and in 24 occasions in 16 tests the plasma cortisol levels did not exceed $20 \mu\text{g}/100\text{ ml}$. The plasma cortisol levels reached 4 to 6 hours

after injection were significantly higher than those found in Addison's disease (p less than 0.0001).

In some patients with secondary adrenal insufficiency a single injection did not secure their differentiation from patients with Addison's disease (16 of 30 tests) and further injections were required to establish the diagnosis. Such injections were administered at 48-hour intervals to 7 patients with Addison's disease and to 11 patients with secondary adrenal insufficiency (fig. 4). Even after 7 injections, the patients with

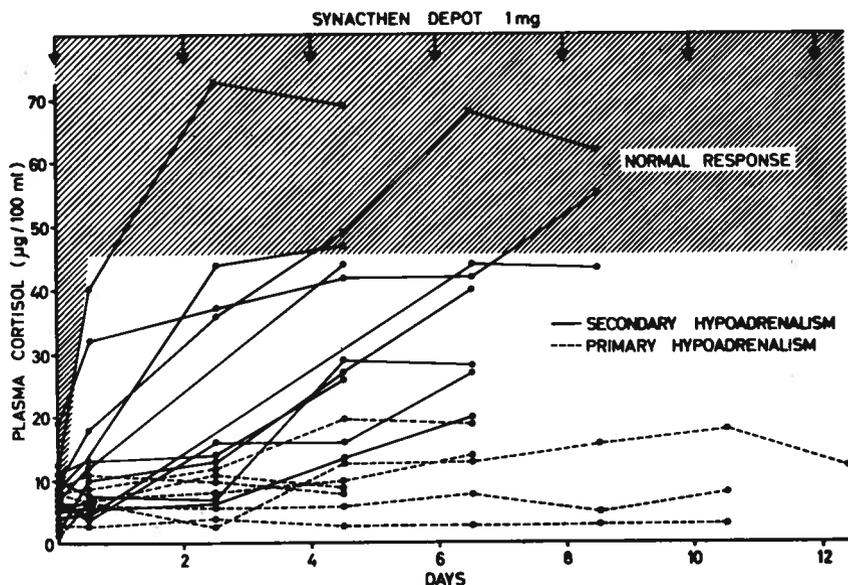


Fig. 4—Response of plasma cortisol to multiple injections of Synacthen Depot administered at 48-hour intervals to 7 patients with primary adrenal insufficiency and to 11 patients with secondary adrenal insufficiency. The normal response is shown by the shaded area

Addison's disease failed to show a significant rise in plasma cortisol. In contrast, with the exception of one patient with secondary adrenal insufficiency who exhibited no rise in plasma cortisol after several injections of Synacthen Depot, all the other patients had a large rise, which served to tell both groups completely apart. Where there is an inadequate response to four injections, more prolonged stimulation is necessary. In Addison's disease, the levels of plasma cortisol never reached 20 $\mu\text{g}/100\text{ ml}$, no matter how many injections were given of Synacthen Depot.

Especial reference must be made to a patient with a non-secreting tumour of the pituitary and the syndrome of amenorrhoea-galactorrhoea, who had five Synacthen Depot tests done when there was a deterioration in the clinical picture (fig. 5). In the first two tests there was a normal adrenal reserve, but some months later the test results were markedly abnormal, reflecting progressive atrophy of the adrenal cortex.

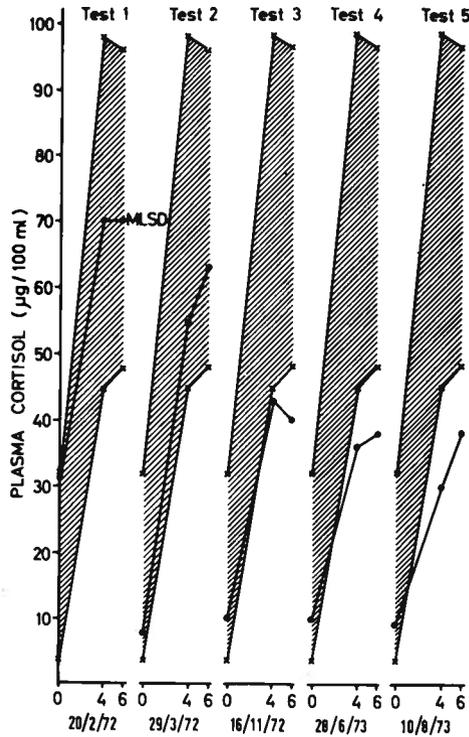


Fig. 5 — Five tests of Synacthen Depot at 4 to 6 hours in patient M.L.S.D. with a huge pituitary tumour and the syndrome of amenorrhoea-galactorrhoea, during a period of clinical deterioration

DISCUSSION

The substance used in this investigation, Synacthen Depot, is a β^{1-24} -corticotrophin depot which has a steroidogenic action which is identical with that of natural corticotrophin. Its absorption is good after intramuscular administration, maximum effect being reached 4 to 16 hours after the injection (Galvão-Teles et al 1971).

In assessing the adrenal response to Synacthen Depot, the use of urine cortisol estimation holds no advantage over early blood level measurement (4 to 6 hours) (Galvão-Teles et al 1971).

In the present paper we confirm our previous results with the Synacthen Depot test (Galvão-Teles et al 1971) but in much larger groups of normals and patients with adrenal insufficiency.

The Synacthen Depot test, with measurement of plasma cortisol by the Mattingly method 4 and 6 hours after injection, is a sensitive test of adrenal reserve: small changes in that reserve can be detected. On the basis of the present results, the normal response can be taken to be the stimulation of plasma cortisol 4 and 6 hours after 1 mg Synacthen Depot to levels of 45 $\mu\text{g}/100\text{ ml}$ or more. A response to Synacthen Depot of less than

20 $\mu\text{g}/100\text{ ml}$ plasma cortisol is consistent with the diagnosis of Addison's disease or severe adrenal atrophy. In such a case, repeated injections of Synacthen Depot are necessary, in order to establish whether the adrenal cortex can respond (as in secondary adrenal insufficiency) or not (as in Addison's disease). Response to the first injection indicates the degree of atrophy or destruction of the cortex, the response to subsequent injections indicating the potential for recovery. Moreover, 4-6 hours after the injection of Synacthen Depot, levels of plasma cortisol of between 20 and 45 $\mu\text{g}/100\text{ ml}$ are found in milder degrees of adrenal atrophy seen in cases of secondary hypoadrenalism.

One of the advantages of this method of assessing adrenal function is that it can be carried out in out-patients.

There was no case of hypersensitivity to Synacthen Depot.

RESUMO

Na insuficiência suprarrenal, quer primária quer secundária, os níveis de cortisol plasmático, cortisol urinário e outros metabólicos podem estar diminuídos ou normais. Assim, compreende-se que a determinação dos níveis basais não tenha qualquer significado diagnóstico. O processo, universalmente utilizado na avaliação da função do córtex suprarrenal, consiste na demonstração da ausência de resposta do córtex ao estímulo pela corticotrofina (ACTH). Neste trabalho a reserva cortical foi determinada pelo doseamento do cortisol plasmático 4 e 6 horas após uma injeção intramuscular de 1 mg de Synacthen Depósito. Foram estudados 87 indivíduos normais (95 provas), 13 doentes com insuficiência suprarrenal primária (26 provas) e 21 doentes com insuficiência suprarrenal secundária (30 provas). Com esta prova do Synacthen Depósito a diferenciação entre normais (cortisol plasmático 4-6 horas após Synacthen Depósito = $62,9 \pm 12,26\ \mu\text{g}/\text{ml}$) e insuficiência suprarrenal, quer primária ($21,3 \pm 12,65\ \mu\text{g}/100\text{ ml}$) foi possível em todos os casos ($p \leq 0,001$). Pelo contrário não foi possível a separação entre os grupos com insuficiência suprarrenal primária e secundária. Neste caso, para o diagnóstico das duas situações foram necessárias múltiplas injeções.

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