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REVIEW

Dose–response aspects in the clinical assessment of the hypothalamo-pituitary-adrenal axis, and the low-dose adrenocorticotropin test*

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Clinical tests of the hypothalamo-pituitary-adrenocortical axis in cases of suspected adrenocortical insufficiency are based almost exclusively on the stimulation of pituitary ACTH release or adrenocortical release of ACTH-dependent steroids. The most widely applied tests of the hypothalamo-pituitary-adrenal axis in clinical practice are the short ACTH injection test (SAT), the insulin hypoglycemia test (IHT), the short metyrapone test (SMT) and the corticotropin-releasing hormone (CRH) test (1). In this review, the reliability of these tests in special clinical settings will be discussed under the aspect of dose–response relationships between plasma ACTH and cortisol in normal man.

Problems with the conventional SAT

If a patient is suspected to suffer from primary adrenal insufficiency (Addison's disease), the test for excluding or strengthening the suspicion is the SAT, comprising the injection (im or iv) of 250 μg of ACTH (1–24) (Synacthen®, Cosyntropin® or Cortrosyn®) and the measurement of plasma or serum cortisol 30 or 60 min after the injection. A basal or post-ACTH plasma cortisol of 550 nmol/l or greater is regarded as safely excluding primary adrenal insufficiency (1–3). In this disorder, basal plasma cortisol is decreased or low-normal and does not respond to ACTH because basal ACTH levels are markedly elevated, thus stimulating the remaining adrenocortical cells to 100% of their maximal secretory capacity (3). In cases of incipient (preclinical) primary adrenal insufficiency, however, it has been shown that measuring basal ACTH levels is even more sensitive than performing the SAT (4). Circulating antibodies directed against the adrenal ACTH receptor may also contribute to refractoriness of the adrenal cortex to exogenous ACTH (5).

Because in severe forms of secondary adrenal insufficiency with low cortisol levels the cortisol response to ACTH may also be absent due to adrenal atrophy and down-regulation of ACTH receptors (6), differentiation between primary and secondary adrenal insufficiency requires the demonstration of elevated plasma ACTH levels in primary insufficiency (3, 4) or the performance of a prolonged ACTH infusion test (1), which results in some increase of cortisol in secondary adrenal insufficiency.

The facts reported up to now are not a matter for debate, but the question of whether the SAT is a good clinical test ("screening test") for any form of adrenal insufficiency and particularly for mild forms of secondary adrenal insufficiency has been unresolved in the past, with several statements for and against made by experienced endocrinologists. The detailed data in this regard have recently been reviewed competently by Grinspoon and Biller (1). Briefly, protagonists of the SAT as a screening test (7–9) found a highly positive correlation in patients with pituitary disease between the results of the SAT and those of the IHT, which is still being regarded by many endocrinologists as the "gold standard" for the detection of any form of adrenal insufficiency. A normal IHT requires a normal activation of hypothalamic corticotropin-releasing mechanisms and normal responses of ACTH and cortisol. Antagonists of the SAT as a screening test (10–16) found discrepancies between the SAT and the IHT or the SMT in patients with pituitary disorders in the sense that the SAT is not sensitive enough to detect mild degrees of secondary adrenal insufficiency. Underdiagnosis of secondary adrenal insufficiency by the SAT may expose some patients to life-threatening complications in situations of severe stress (e.g. major surgery) when intermittent steroid replacement therapy would have been necessary. Antagonists and protagonists agree in the view that the SAT is not indicated in cases of suspected acute secondary adrenal insufficiency (e.g. a few weeks after pituitary surgery), because adrenal atrophy (the main reason for adrenal hyporesponsiveness to ACTH in SAT) did not have sufficient time to develop (1).

The SAT in the light of dose–response relationships between plasma ACTH and cortisol

Early studies by Landon et al. (17) made it clear that the infusion of 4 μg per hour of ACTH(1–24) was sufficient to stimulate the adrenal cortex maximally. In spite of this observation, which obviously fell into oblivion until recently, the SAT has been performed for about 30 years with a dose of 250 μg of ACTH(1–24). Other authors (18, 19) confirmed the results of Landon et al.

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(17), but studies with intact human ACTH(1–39) and measurement of plasma ACTH levels after injection have not been performed. Could the marked overdose of ACTH be a cause of the relative insensitivity of the SAT in patients with mild secondary adrenal insufficiency?

For investigating acute dose–response relationships between plasma ACTH and cortisol in normal man, we injected synthetic human ACTH(1–39) subcutaneously into eight normal male subjects and measured the course of plasma ACTH and cortisol with sensitive radioimmunoassays within the next 2 h (20). Doses of ACTH between 2.5 and 30 μg were used in a randomized order. The studies were performed in the afternoon, when endogenous ACTH and cortisol levels are relatively low, and each subject received five different doses of ACTH on different days with a time interval of at least 3 days. In order to compare the response of the adrenal to injected ACTH(1–39) with that to ACTH released by iv administration of human CRH, the experimental subjects also received 30 and 100 μg of hCRH on different days. Mean peak plasma ACTH levels were then plotted against mean peak increments of plasma cortisol, as shown in Fig. 1. Mean basal plasma cortisol levels in this study were around 200 nmol/l. There is a very steep initial segment of the dose–response curve. If we define the cortisol response to 30 μg of ACTH (plasma ACTH between 300 and 350 ng/l) as 100%, it is obvious that a near-maximal cortisol response (77%) was obtained with plasma ACTH levels as low as 60–70 ng/l. These levels are slightly higher than normal plasma ACTH in the morning (5–45 ng/l). In a previous study (21) we measured plasma ACTH in several normal subjects about 10 min after im injection of 250 μg of ACTH(1–24) and found it to be invariably higher than 2000 ng/l (ACTH-level data not published previously). Thus, in the course of the SAT, the adrenal cortex is exposed to excessively high ACTH levels. Although the plasma ACTH–cortisol dose–response curve is rather flat at ACTH levels greater than 100 ng/l in the normal, it is possible that a partially atrophic adrenal with a decreased number of ACTH receptors per cell in patients with secondary adrenal insufficiency exhibits a dose–response curve that is different from that of a normal gland in the sense that an insufficient cortisol response to ACTH levels in the physiological range can be overcome by a huge overdose of ACTH. These considerations led to clinical tests with greatly reduced dosages of ACTH.

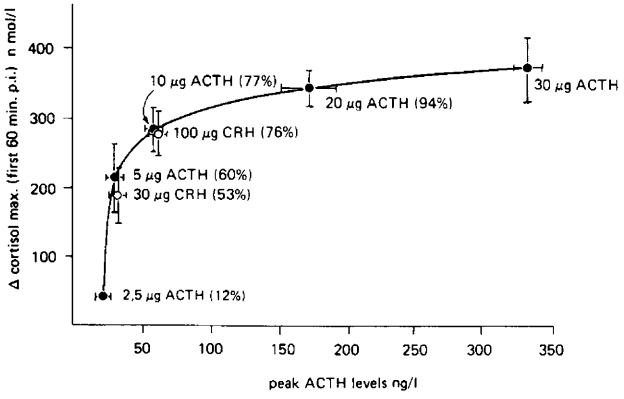

Fig. 1. Dose–response relationships between plasma ACTH and delta cortisol after sc injection of different dosages of human ACTH(1–39) or iv injection of 30 and 100 μg of human CRH. The peak response of ACTH and cortisol to 30 μg of ACTH is arbitrarily defined as 100%. Mean values ± SEM are shown. To convert ACTH values in ng/l to pmol/l, multiply by 0.22. To convert cortisol values in nmol/l to μg/dl, multiply by 0.036 (Ref. 20).

Low-dose SAT

Dickstein et al. (14) and Broide et al. (22) from Haifa recently developed a low-dose ACTH(1–24) test (LD-SAT). In normal subjects, the cortisol response 20 min after injection of 0.5 μg per 1.73 m^2 of ACTH was not different from that after a single dose of 250 μg of ACTH, while 45 min after injection the cortisol response was significantly lower (and falling) after 0.5 μg of ACTH than after 250 μg (still rising). When results of the conventional SAT and of the LD-SAT in 46 asthmatic children and young adults who chronically inhaled topically active glucocorticoids (budesonide or beclomethasone diproprionate) were compared with those in 33 age-matched controls, the following differences emerged: only one patient had a subnormal cortisol response in the 250-μg SAT, while 16 patients failed to reach a peak cortisol response to 500 nmol/l or greater in the LD-SAT. Such a response was failed by only one of the control subjects in the LD-SAT. An additional criterion of normality in the LD-SAT was an increment of cortisol from baseline by at least 200 nmol/l. Patients with a subnormal cortisol response in the LD-SAT had significantly lower urinary free cortisol excretion levels (with a large overlap to normal) than patients with a normal response or control subjects (Fig. 2). These data seem to indicate that the LD-SAT can detect mild forms of secondary adrenal insufficiency that escape detection by the conventional SAT.

Another article from Israel (23) comes to similar conclusions: Tordjman et al. compared the cortisol response to 250, 5 and 1 μg of ACTH(1–24) in seven normal subjects (group 1), 10 patients with pituitary macroadenomas and pathological steroid responses in the IHT or SMT (group 2) and nine patients with pituitary macroadenomas and normal IHT and SMT results (group 3). Basal morning cortisol levels in the three groups were similar, and no patient required regular hydrocortisone substitution. A plasma cortisol response to a level 497 nmol/l (18 μg/dl) or greater was regarded as "pass" in each variant of the SAT and in the IHT. Groups 1 and 3 were essentially indistinguishable from each other with the three types of SAT. However, the cortisol responses to 1 μg were at all time points

significantly lower than the responses to the higher doses of ACTH, in variance to the findings of Dickstein et al. (14) and Broide et al. (22). No subject of group 2 passed the 1-μg ACTH test as normal, while 7/10 group 2 patients would have passed the 5-μg ACTH test and 9/10 the 250 μg test as normal. The data presented in this paper support the view that a LD-SAT seems worthy of being evaluated extensively for the detection of milder forms of secondary AI because it is easy to perform, has no risk for the patient and is cheaper than most other tests. Similar results with a LD-SAT were reported recently by Rasmuson et al. (24).

Dose–response aspects of the CRH test

The CRH test is a test of secondary adrenal insufficiency and, in conjunction with the IHT, a valuable tool for the differential diagnosis between secondary and tertiary (hypothalamic) adrenal insufficiency (25). The test procedure has not been well standardized. Some groups perform the test in the morning and others in the late afternoon when endogenous ACTH and cortisol is low. Some groups inject 1 μg/kg body weight of human or ovine CRH iv and others use a standard dose of 100 μg irrespective of body weight (25–27). As an example of normal test results in 50 healthy adults, the data of Schlaghecke et al. (26) are of interest. They injected 100 μg of hCRH between 8.00 and 9.00 a.m. and found an increase from a basal cortisol level of 333 ± 101 SD to a maximum of 568 ± 188 nmol/l. The ACTH level increased from 24 ± 17 to a maximum of 49 ± 26 ng/l.

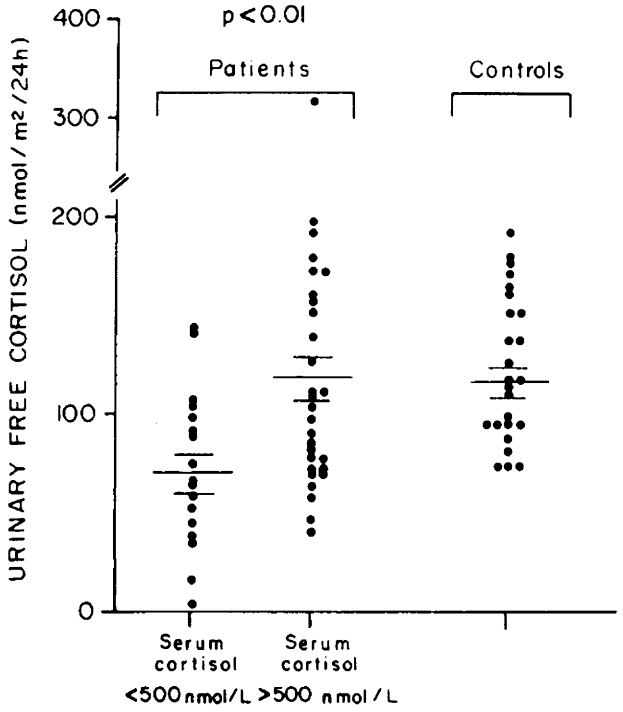

Fig. 2. Urinary free cortisol excretion in normal controls and in children or young adults (patients) with asthma treated with inhaled beclomethasone or budesonide for more than 6 months. Sixteen out of 46 patients (left column), but only one out of 33 controls, failed to respond to 0.5 μg of ACTH per 1.73 m^2 with an increase of serum cortisol to >500 nmol/l. These 16 patients had significantly lower urinary free cortisol levels than controls or patients responding normally to 0.5 μg of ACTH per 1.73 m^2 (medium column). All patients except one had normal serum cortisol responses (>500 nmol/l) to the standard dose of 250 μg of ACTH(1–24). To convert serum cortisol values in nmol/l to μg/dl, multiply by 0.036. To convert urinary free cortisol values in nmol/24 h to μg/24 h, multiply by 0.36 (Ref. 22).

As shown in Fig. 1, injection of 30 and 100 μg of hCRH into normal men leads to plasma ACTH peaks of about 32 and 57 ng/l, respectively, and the cortisol responses to these increments in ACTH falls exactly into the dose–response curve that has been constructed from results of the ACTH(1–39) injection study. Thus, bolus injections of CRH seem to stimulate cortisol exclusively through ACTH and not by other cleavage products of proopiomelanocortin. Orth et al. (28) tested the response of ACTH and cortisol to a wide range of oCRH dosages in normal man. The highest dose (30 μg/kg body weight) that caused unpleasant side effects did not stimulate plasma ACTH to mean levels greater than 80 ng/l. The ACTH response to 1 μg/kg body weight in Orth's study (28) was about 40–50 ng/l. The cortisol response to the highest dose of CRH was only slightly greater than that to 1 μg/kg. This would fit well into the flattening part of our dose–response curve (Fig. 1). The moderate response of plasma ACTH to a very large dose of CRH is probably due to a strong feedback of rising cortisol levels on the corticotroph cells of the pituitary, where glucocorticoids markedly blunt the ACTH response to CRH (25). Thus, even very high doses of CRH induce only a submaximal response of cortisol, while the standard tests for secondary adrenal insufficiency (the IHT and the SMT) lead to plasma ACTH levels of greater than 150 ng/l (29, 30) and to an almost maximal acute cortisol response. Nevertheless, the results of the CRH test and the IHT correlated remarkedly well ($r = 0.82$) in a large number of patients on long-term glucocorticoid therapy, although plasma ACTH and cortisol responses in the IHT were more pronounced than in the CRH test (26). Furthermore, the CRH test has been an extremely valuable research tool in studying the cortisol–ACTH feedback in pathological states like Cushing's disease, anorexia nervosa and hypercortisolism associated with depression (25, 31).

The SMT in the light of dose–response relationships

The SMT and the IHT have been the standard tests for the detection of secondary adrenal insufficiency. The advantage of the SMT over the IHT is that only one blood sample is required and it can be performed when

the IHT is contraindicated, e.g. in patients with coronary heart disease or at risk for cerebral convulsions. The SMT comprises the administration of 30 mg/kg body weight of metyrapone at midnight and the measurement of plasma-11-deoxycortisol at 08.00 h the following morning (32). Metyrapone blocks the adrenal enzyme 11β-hydroxylase, thereby inhibiting the formation of cortisol and stimulating the secretion of ACTH and the cortisol precursor 11-deoxycortisol. In normal subjects, 11-deoxycortisol rises after metyrapone administration from almost undetectable levels to 200 nmol/l or greater (12, 32, 33). If plasma ACTH is measured in addition, its levels are >150 ng/l in almost every normal subject (29). Feek et al. (34), using the SMT with the measurement of plasma ACTH exclusively, made the interesting observation that in a group of patients with proven pituitary disorders, the SMT with ACTH measurement was more sensitive for discovering mild forms of secondary adrenal insufficiency than the IHT with the measurement of plasma cortisol. When we had evaluated our ACTH–cortisol dose–response study (20), we interpreted the data of Feek et al. (34) in the following way: ACTH is stimulated in the SMT as well as in the IHT vigorously to levels >150 ng/l (29, 30). Because the ACTH–cortisol dose–response curve becomes flat at ACTH levels greater than 60–70 ng/l (Fig. 1), a subnormal ACTH response to 50 or 60 ng/l in a patient with mild secondary adrenal insufficiency in either test may still be able to stimulate 11-deoxycortisol or cortisol to above the lower limit of normal. The subnormal response of the hypothalamic-pituitary unit could, therefore, be detected by measuring ACTH but not by steroid measurement. We tested this hypothesis in 20 endocrinologically healthy subjects (group 1) and in 95 patients with proven pituitary disease, 25 of whom had already been on daily hydrocortisone replacement therapy because of more severe secondary adrenal insufficiency (group 2b), while the others ($n = 70$) were not substituted (group 2a). A standard SMT with the measurement of plasma 11-deoxycortisol plus ACTH was done in each subject. Patients of group 2b (between 10 and 25 mg of hydrocortisone per day) observed a therapy-free interval of at least 24 h before the test (35). The results are shown in Fig. 3. The relationship between ACTH and 11-deoxycortisol in these 115 subjects resembles the ACTH–cortisol dose–response curve of Fig. 1. All control subjects of group 1 had normal ACTH responses to >150 ng/l and normal 11-deoxycortisol responses to >200 nmol/l. All patients of group 2b had subnormal ACTH and 11-deoxycortisol responses. Twenty-three patients of group 2a had completely normal ACTH and 11-deoxycortisol responses in the SMT. In these patients, secondary adrenal insufficiency could be excluded. Twenty-one other patients of group 2a (30%) had subnormal 11-deoxycortisol responses, but 47 patients (67%) had subnormal ACTH responses. Thus, 37% of the 70 patients in group 2a with pituitary disease had subnormal ACTH responses with a normal

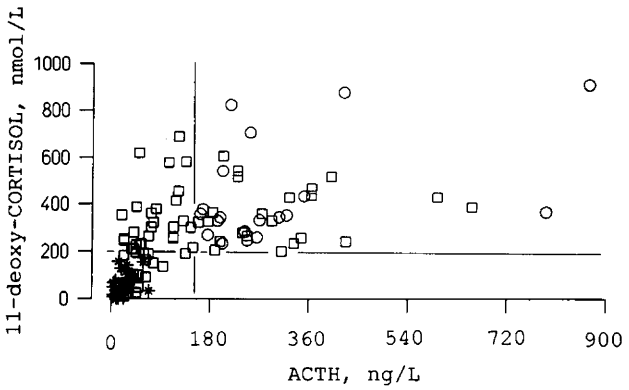

Fig. 3. Relationship between plasma ACTH and 11-deoxycortisol at 08.00 h after administration of 30 mg/kg body weight of metyrapone at midnight (○) 20 endocrinologically healthy subjects (group 1); (□) 70 patients with proven pituitary disease not on hydrocortisone substitution so far (group 2a); (*) 25 patients with proven pituitary disease receiving regular hydrocortisone substitution (group 2b). Hydrocortisone was omitted at least 24 h before the test. The lines within the graph mark the lower limits of ACTH response (150 ng/l) and of 11-deoxycortisol response (200 nmol/l) to metyrapone. To convert ACTH values in ng/l to pmol/l, multiply by 0.22. To convert 11-deoxycortisol values in nmol/l to μg/dl, multiply by 0.035 (Ref. 35).

11-deoxycortisol, i.e. measurement of ACTH was more sensitive than 11-deoxycortisol for detecting mild dysfunction of the hypothalamo-pituitary-adrenal axis.

Is the IHT also affected by the dose–response ideology?

Because ACTH rises to about the same high levels in the IHT as in the SMT (29), as mentioned before, it could be predicted that the IHT with measurement of plasma cortisol only would be blind, like the SMT with 11-deoxycortisol measurement only, to detect milder forms of hypothalamo-pituitary-adrenal insufficiency. To test this supposition, we measured intact plasma ACTH (IRMA method, kit from Nichols Institute, San Juan Capistrano, CA) and cortisol in the scope of the IHT (0.1–0.15 IU insulin/kg body weight) in 30 consecutive patients with pituitary tumors (pre- or postoperatively, excluding patients with Cushing's disease) and in six healthy young men and plotted the peak levels of ACTH against those of cortisol (Fig. 4). Only tests with a fall of blood glucose to <2.2 mmol/l (<40 mg%) and a clinical response (sweating, tachycardia) were used. It is evident that the relationship between the responses of ACTH and cortisol resembles that shown in Figs 1 and 3. The six control subjects and seven patients had completely normal ACTH and cortisol responses to hypoglycemia. Nine patients had a normal cortisol response in spite of a subnormal ACTH response, and 13 patients had subnormal ACTH and cortisol responses. Thus, in 9 of 30 patients with pituitary tumors (30%) a mild dysfunction of the hypothalamo-pituitary-

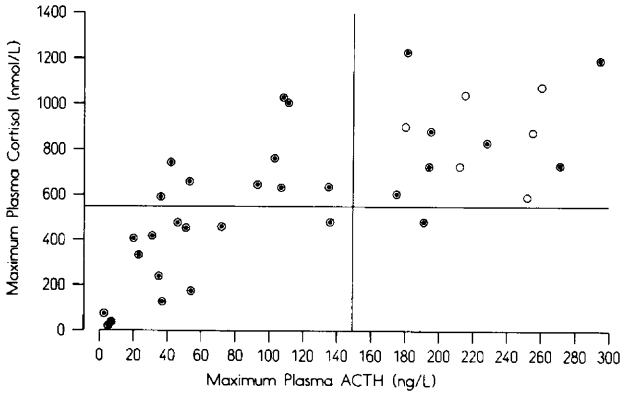

Fig. 4. Relationship between maximum plasma ACTH and cortisol responses to insulin-induced hypoglycemia (blood glucose <2.2 mmol/l or <40 mg/dl) in six normal subjects (○) and in 30 patients with pituitary tumors pre- or postoperatively (●). The lines within the graph mark the lower limits of ACTH response (150 ng/l) and of cortisol response (550 nmol/l) to hypoglycemia. Plasma ACTH (ng/l) × 0.22 = pmol/l; plasma cortisol (nmol/l) × 0.036 = μg/dl.

adrenal axis was only detected by measuring ACTH, not by cortisol, in the IHT. The observation by Feek et al. (34) mentioned above that the SMT with ACTH measurement is more sensitive for detecting mild hypothalamo-pituitary-adrenal insufficiency than the IHT with cortisol measurement, is thus easily explained by the fact that a normal ACTH response in the SMT as well as in the IHT is markedly greater than necessary for stimulating adrenocortical steroid secretion to give a response in the low-normal range. Lindholm et al. (36), 17 years ago, reported on a "discrepancy between ACTH and cortisol responses to insulin-induced hypoglycemia" in 26 healthy subjects. They found no correlation between peak plasma ACTH and cortisol levels in the IHT, cortisol levels being equally high whether the ACTH response was low-normal or high-normal. They interpreted their data as showing an "overcapacity of ACTH secretion in comparison to the secretory capacity of the adrenal cortex" or, as we would put it now, the flatness of the dose–response curve at supraphysiological ACTH levels.

There are few reports in the literature on failures of the IHT to detect secondary adrenal insufficiency. Tsatsoulis et al. (37) reported on six patients previously treated by external pituitary irradiation presenting with excessive tiredness, who had normal cortisol responses in the IHT ($n = 4$) or in the glucagon test ($n = 2$), but low urinary free cortisol and rather low plasma cortisol profiles. These patients profited markedly from regular glucocorticoid replacement therapy. It is very likely that the abnormality of the hypothalamo-pituitary-adrenal axis would have been discovered in these patients if ACTH had been measured after insulin administration in addition to plasma cortisol.

Conclusions and clinical relevance of modified hypothalamo-pituitary-adrenal tests

Published reports and the data presented here have shown that the conventional high-dose SAT is not sensitive enough to reveal mild forms of secondary adrenal insufficiency and even preclinical primary adrenal dysfunction (4). There are only a few reports on the insensitivity of the IHT in the literature, and it can therefore be assumed that the scotoma in the eye of the IHT is smaller and less relevant than that of the high-dose SAT. The strategy to overcome the problems with the SAT is to use much smaller dosages of ACTH (LD-SAT), which probably lead to plasma ACTH levels that stimulate the adrenal cortex in the steep or flattening part of the dose–response curve, as shown in Fig. 1. Because such tests carry no risk for the patient, and because the measurement of plasma cortisol is relatively cheap, they should be evaluated carefully in several endocrinological centers on larger populations of controls and of patients who are at risk of having or developing secondary adrenal insufficiency. The studies thus far published on the LD-SAT are encouraging, but the number of patients examined in each single study is too small to allow final conclusions regarding the sensitivity and specificity of the test. Furthermore, a consensus on the ACTH dose in the LD-SAT and on the "pass" criterion is required (38, 39).

If symptomatic primary adrenal insufficiency is the suspected diagnosis, the high-dose SAT should be used further together with basal ACTH measurement (3, 4). An established valuable test of secondary and tertiary adrenal insufficiency is the CRH test, which also caries no risk for the patient but is more expensive than the LD-SAT, especially if serial ACTH measurements are intended. If the LD-SAT was able to detect mild secondary adrenal insufficiency with high reliability, then the performance of IHT and SMT may be restricted to a minority of patients in whom special questions arise or in whom insulin-induced hypoglycemia is indicated for other reasons. In such cases, measurement of plasma ACTH in addition to cortisol may give valuable additional information that justifies the costs of ACTH measurement and the trouble of transporting chilled blood or frozen plasma samples to the laboratory. For the SMT, only one ACTH sample is required. We have used the SMT with ACTH measurement routinely for several years without problems and with a significant gain of information. We give patients with a normal 11-deoxycortisol response, but a clearly subnormal ACTH response, a steroid card with the recommendation to substitute glucocorticoids in stressful situations. Closer follow-up is also recommended in such patients.

Finally, it has to be pointed out that the dose–response relationship presented in Fig. 1 is not applicable to patients with hyperplastic adrenal glands (e.g. Cushing's disease) or to those exposed to prolonged stress, e.g. intensive-care patients. In the latter, very

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