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II. Medizinische Klinik der Universität München, Deutschland

QUANTITATIVE ASSAY OF THE SUPPRESSIVE EFFECT OF SYNTHETIC CORTICOIDS IN MAN

Evaluation of the circadian rhythm of serum cortisol after single oral doses of fluocortolone and prednisolone

By

*N. Boss, F. Kluge, O. A. Müller, C. R. Pickardt and P. C. Scriba**

ABSTRACT

The suppressive effect of synthetic corticoids on the human hypothalamic-pituitary-adrenal axis was analyzed by determining the integral differences of the circadian serum cortisol levels between control subjects (N = 25) and subjects receiving different single oral doses of prednisolone (N = 29) or fluocortolone (N = 36). Linear log dose-response curves were obtained by this procedure with the indices of precision for prednisolone of $\lambda = 0.221$ and for fluocortolone of $\lambda = 0.149$. With this method it is possible to compare the suppressive effect of different corticoids. The relative suppressive potency of corticoids should not be expressed by ratios or factors, but rather by log dose-response curves of the type: response = b · log dose + a.

This study was conducted in order to develop a procedure for quantitating the suppressive effect of single oral doses of synthetic corticoids on the hypothalamic-pituitary-adrenal axis in man, since the available data in the literature were considered to be unsatisfactory (*Hedner* 1967; *Radvila et al.* 1969; *Hochheuser et al.* 1969; *Bethge* 1970). *Hedner* (1967) administered orally four synthetic corticoids at four equipotential dose levels at midnight and considered the reduction in the 8.00 a. m. plasma corticosteroid values as the

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suppressive effect. Rectilinear log dose-response curves were obtained with indices of precision from $\lambda = 0.19$ to $\lambda = 0.39$. *Radvila et al.* (1969) followed the circadian variation of serum 11-hydroxycorticosteroids after oral corticoid administration, but only single dose levels of dexamethasone and triamcinolone were used.

The present study was performed in order to obtain linear log dose-response curves for prednisolone and fluocortolone with reasonable indices of precision (λ). Therefore various dose levels of these corticoids were administered orally and the curves of the circadian serum cortisol values were subsequently analyzed until the intersection with the normal circadian cortisol profile. By this means, both the degree and duration of the suppression of the hypothalamic-pituitary-adrenal axis by a single corticoid dose could be taken as response.

Preliminary results of this study have been reported (*Boss et al.* 1970; *Scriba et al.* 1970*a*).

MATERIAL AND METHODS

The subjects under study were hospitalized for various non-acute, non-endocrine diseases. Subjects were either selected for the control group or suppressed with only one single dose of the synthetic corticoids used, usually during convalescence shortly before being discharged from the hospital. The control group (N = 25 males) had an average weight of 73.0 ± 10.6 kg and were 41.7 ± 16.1 years of age (mean ± SD). The prednisolone group (N = 29 males) and the fluocortolone group (N = 36 males) had average weights of respectively 75.2 ± 11.1 kg, and 70.1 ± 11.7 kg, and were respectively 41.0 ± 14.0 and 41.5 ± 15.5 years of age. The two totally adrenalectomized patients were both females (M. M., 43 years; H. M., 46 years). Adrenalectomy in these cases was performed several years before this study because of Cushing's syndrome with bilateral adrenal hyperplasia.

Prednisolone (Pregna-1,4-dien-11β,17α,21-triol-3,20-dione) and fluocortolone (6α-fluoro-16α-methyl-pregna-1.4-dien-11β,21-diol-3,20-dione) were given orally as tablets (5 mg) at 7.00 a. m. in doses indicated in Figs. 3 and 4. Blood was collected in polystyrol tubes at the intervals indicated in Figs. 1, 5 and 6; the serum was stored at *–20°C until determination.*

Serum cortisol was determined fluorimetrically as previously described (*Kluge et al.* 1970; *Scriba et al.* 1970*b*). The suppressive effect of prednisolone and fluocortolone on the circadian rhythm of serum cortisol was determined as shown in Figs. 1 and 2.

Statistical analysis was performed as indicated throughout the text (*Wallis & Roberts* 1969; *Gaddum* 1953). The authors are indebted to Dr. P. Kuhbier, München, for his valuable help in the statistical evaluation.

RESULTS

The suppressive effect of various single oral doses of synthetic corticoids administered at 7.00 a. m. was determined by calculating the integral differences

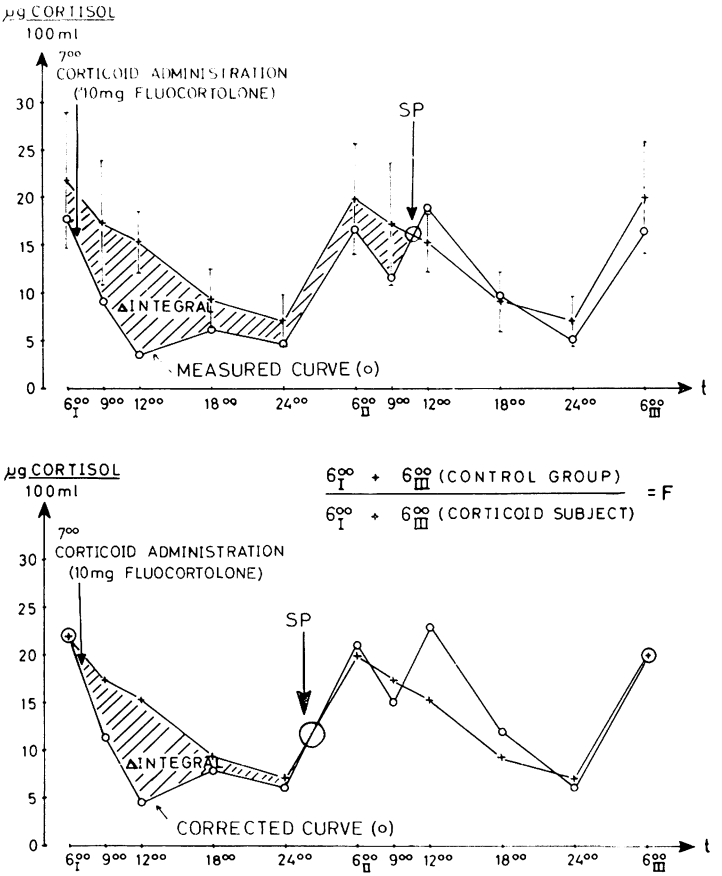

Fig. 1 a and b.

Example of the suppression of circadian serum cortisol levels by a single oral dose of corticoid, i. e. fluocortolone at 7.00 a. m. The circadian rhythm of serum cortisol levels of the controls (mean ± SD, N = 25) for 48 h is represented by the upper curve in both parts of Fig. 1 (+).

a) *Measured curve.*

The serum cortisol levels measured in a subject given 10 mg fluocortolone at 7.00 a. m. are shown as an example by the lower curve (o). Since the values of the corticoid subject at 6.00 a. m. on the first and third day are different from the mean values of the controls, each cortisol value of the corticoid treated subjects, receiving fluocortolone or prednisolone, had to be corrected.

b) *Corrected curve.*

The corrections for the initial 6.00 a. m. and for subsequent values were performed by multiplying the measured cortisol values of each corticoid treated subject (here fluocortolone) by a factor F, derived from dividing the 6.00 a. m. values of the first and third day of the control group by the 6.00 a. m. values of the first and third day of each corticoid treated subject. Analogous corrections were then applied to the cortisol levels of each corticoid treated subject receiving either fluocortolone or prednisolone. The corrected levels of serum cortisol of the subject receiving 10 mg fluocortolone are shown in the lower curve (o). The hatched area between the control and the corrected corticoid curve was calculated as the difference between the integrals of the two curves (Δ integral = suppressive effect) from the start at 6.00 a. m. until the intersection (SP).

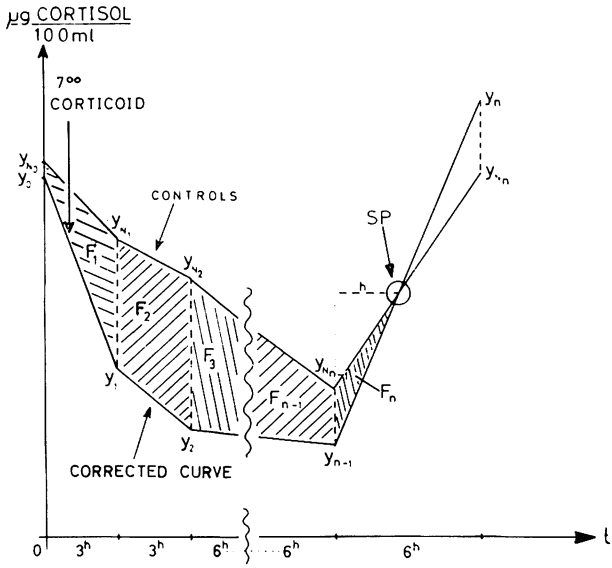

Fig. 2.

Schematic presentation of the calculation of the suppressive effect of a single oral dose of corticoid, i. e. fluocortolone or prednisolone.

The difference of the integrals $(\Delta \int_0^{SP})$ of the circadian cortisol curves of the controls (y_N) and of a corticoid subject (y) from the start (t = 0) until the intersection (SP) was calculated as the sum of the single areas F_1 to F_n:

$$\Delta \int_0^{SP} = F_1 + F_2 + \ldots\ldots + F_{n-1} + F_n$$

$$F_1 = \left[(y_{N_0} - y_0) + (y_{N_1} - y_1) \right] \cdot \frac{t}{2}$$

$$F_2 = \quad \vdots$$

$$F_{n-1} = \left[(y_{N_{n-2}} - y_{n-2}) + (y_{N_{n-1}} - y_{n-1}) \right] \cdot \frac{t}{2}$$

$$F_n = (y_{N_{n-1}} - y_{n-1}) \cdot \frac{h}{2}$$

h was derived from geometric formulae. The calculation was performed with a computer (Olivetti Programma 102). The authors are indebted to Dr. Peter v. Breitenlohner, München, for setting up the programme for the computer.

between the circadian cortisol levels in the controls and in the subjects receiving corticoids. There was a linear log dose-response relationship between the log dose of prednisolone, or of fluocortolone, and the suppressive effect $\Delta \int_0^{SP}$. This was shown for the absolute dose of corticoid in mg (Fig. 3) and also for μg per kg body weight (Fig. 4). The correlation coefficients (r) and the indices of precision (λ) for both prednisolone and fluocortolone are satisfactory (Figs. 3 and 4), in view of the fact, that the model used might be considered as a method of bioassay in the human subject.

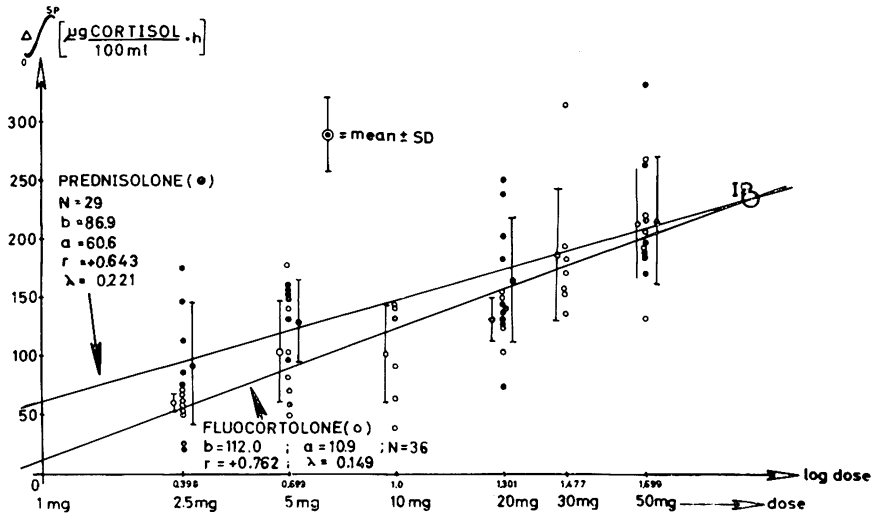

Fig. 3.

Suppressive effect ($\Delta \int_0^{SP}$) of prednisolone (●) and of fluocortolone (o) plotted as log dose in mg.

Single values and means ± SD of the suppressive effect, calculated as described in Fig. 2, are shown for the log dose of corticoids, given as a single dose at 7.00 a.m. Linear log dose-response curves were calculated from $y = bx + a$ with

the regression: $b = \dfrac{\Sigma xy - N\bar{x}\bar{y}}{\Sigma x^2 - N\bar{x}^2}$,

the suppression of the fictive dose of 1 mg:

$$a = y \text{ for } x = 0,$$

the correlation: $r = \dfrac{\Sigma xy - N\bar{x}\bar{y}}{\sqrt{(\Sigma x^2 - N\bar{x}^2)(\Sigma y^2 - N\bar{y}^2)}}$,

and the index of precision: $\lambda = \dfrac{s_b}{b}$; where $s_b = \dfrac{s_y}{s_x}\sqrt{\dfrac{1-r^2}{n-2}}$.

The two dose-response curves intersect at IP.

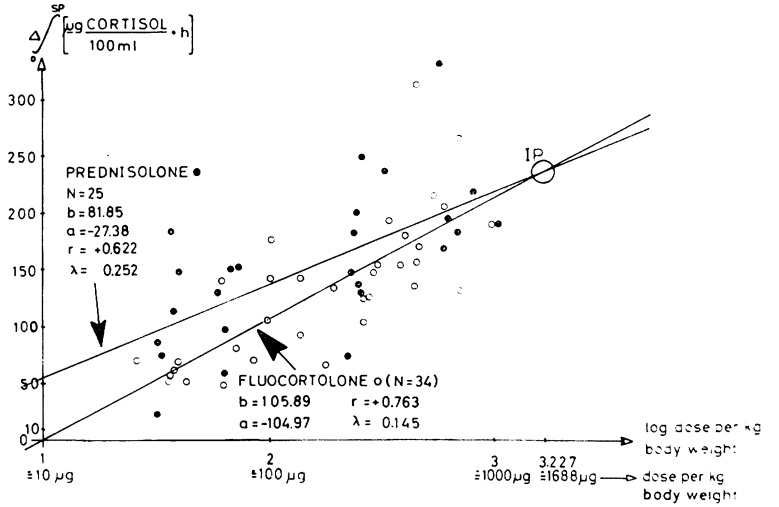

Fig. 4.

Suppressive effect ($\Delta \overset{SP}{\underset{o}{\backslash}}$) of prednisolone (●) and of fluocortolone (o) plotted as log dose in µg per kg body weight.
The linear log dose-response curves were calculated as described in Fig. 3.

Apparently the relative suppressive potencies of the two corticoids compared cannot be characterized by a simple factor or ratio (*Hedner* 1967). Instead, the suppressive effect has to be expressed by dose-response curves $\Delta \overset{SP}{\underset{o}{\backslash}} = b \cdot \log$ dose + a (Figs. 3 and 4). The two curves obtained for prednisolone and for fluocortolone are slightly different with regard to their regressions (b). The difference between the regressions is hardly of statistical significance when the dose is plotted (Fig. 3) in mg ($P < 0.15$), or (Fig. 4) in µg per kg body weight ($P < 0.2$).

However, the assumption that the two curves intersect at IP (Figs. 3 and 4) is supported by the fact, that the values for a, which denote the suppression at doses of respectively 1 mg, and 1 µg per kg body weight, are significantly different ($P < 0.05$; prednisolone: N = 29, fluocortolone: N = 36). The log dose-response curves do not cross at the intersection of the ordinate and abscissa, since the log of a dose of zero would be indefinite.

No significant difference was found for the various corresponding groups receiving prednisolone or fluocortolone in doses ranging from 2.5 to 50 mg (Figs. 3 and 4). On the other hand, a dose as low as 2.5 mg of prednisolone or

of fluocortolone suppressed the cortisol levels significantly as compared with the controls ($P < 0.0125$).

The slight difference in the regressions (b) of the two dose-response curves for prednisolone and fluocortolone may be derived from Fig. 5. The suppressive effect of prednisolone in lower doses, e. g. 20 mg, is more rapid in onset and also more marked than the effect of fluocortolone, whereas the effect of fluocortolone appears to last somewhat longer. These differences in onset of the suppressive effect diminish with higher doses. However, the longer duration of the fluocortolone effect may possibly cause a more pronounced suppressive effect of fluocortolone at doses above approximately 100 mg (Figs. 3 and 4).

Neither prednisolone nor fluocortolone show any fluorescence in the alcohol-sulphuric acid reagent. However, an important question to be answered is, whether the metabolites of these synthetic corticoids interfere with the fluorimetric determination. The fluorescence of serum from adrenalectomized patients (Fig. 6) was therefore determined for 48 h after the administration of 50 mg prednisolone, and fluocortolone respectively. No evidence of any such fluorescent metabolites was obtained.

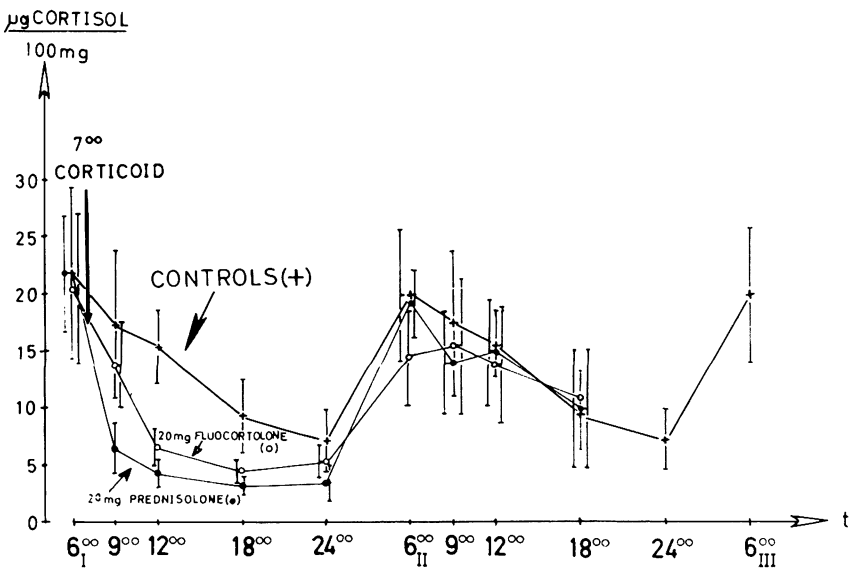

Fig. 5.

Dynamics of the suppression induced by prednisolone and by fluocortolone. The mean values ± SD of circadian serum cortisol levels of controls (N = 25) and of subjects receiving 20 mg prednisolone (N = 9) or 20 mg fluocortolone (N = 5) at 7.00 a. m. are shown.

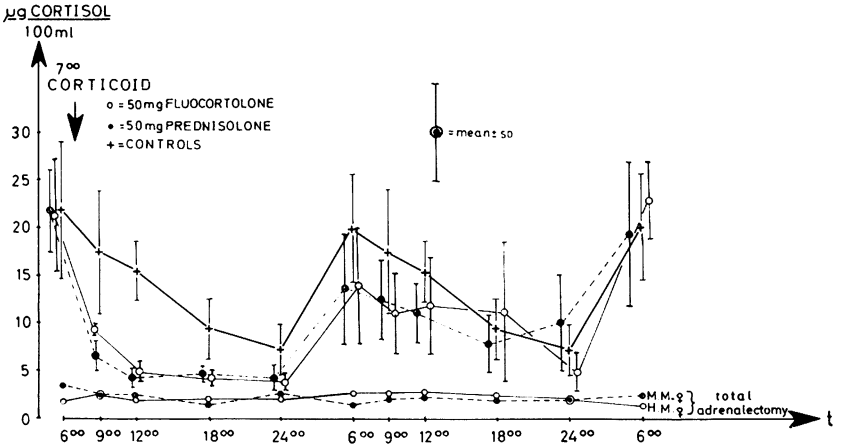

Fig. 6.

Basic fluorescence in totally adrenalectomized patients after 50 mg prednisolone or fluocortolone at 7.00 a. m.

The circadian cortisol curve of normal controls (+) and normal subjects receiving 50 mg prednisolone (●) or fluocortolone (o) were compared with the fluorimetric values of serum from totally adrenalectomized patients (M.M., H.M.). Totally adrenalectomized patients were taken off cortisol replacement therapy 18 h before the first serum cortisol determination (6.00 a. m.) and received 50 mg of prednisolone (●) or fluocortolone (o) at 7.00 a. m. No evidence for the presence of any fluorescent metabolites of these synthetic corticoids was obtained. The values were close to the basic fluorescence of »cortisolfree« serum (1–2 µg/100 ml) in this assay (*Kluge et al.* 1970).

DISCUSSION

The proposed method for calculating the integral differences of serum cortisol levels between controls and subjects receiving synthetic corticoids, make it possible to quantitate the suppressive effect of corticoids in man. It shows that any analysis of the suppressive action of different corticoids should take into account both the degree and duration of suppression. This conclusion is thought to be valid despite the recent observation of *Hellman et al.* (1970), that cortisol is secreted episodically.

The approach presented in this paper may provide a means of comparing the suppressive potency of various corticoids. The relative potencies cannot be expressed by simple ratios or factors (*Hedner* 1967). Rather, dose-response curves have to be obtained as demonstrated under Results, since the relation between the potencies is not constant throughout the whole range of doses administered. It would appear to be justifiable to look for analogous dose-response relationships for other corticoid effects in man, e. g. diabetogenic, anti-inflammatory, ulcerogenic and other actions of corticoids.

It remains, however, an open question whether the suppressive effects of long

term corticoid therapy and of single oral corticoid doses, as described in this paper, are the same for all synthetic corticoids.

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