Radioimmunological Determination of Plasma Cortisol

MURPHY et al.[1] and afterwards other investigators[2,3] have described a protein-binding assay of plasma cortisol. The principle of that assay is the competition for transcortin of the cortisol contained in the plasma sample and a known amount of tritiated cortisol. Plasma having a high concentration of transcortin (e.g. from pregnant women) is used for the assay. However, the binding capacity of transcortin is not specific for cortisol, since corticoids, such as corticosterone, compound S, and others, are also bound by that plasma-protein fraction. Therefore, under pathological conditions in which the concentration of those corticosteroids in the plasma is elevated, the results of the protein-binding assay are ambiguous. In view of the higher specificity of steroid antibodies, a radioimmunoassay for cortisol has been developed.

White New Zealand rabbits were immunized with a cortisol-21-hemisuccinate bovine serum albumin complex prepared according to the procedure described by ERLANGER et al.[4]. 1 mg of the antigen was suspended in 0.1 ml of saline and 0.5 ml of complete Freund's adjuvant (DIFCO) immediately before i.m. injection. The same injections were repeated every 2 to 4 weeks. After 6 months, 6 out of 8 immunized rabbits had high antibody titers.

Table I. Cross reactions (in %)

Aldosterone	0.36 (3)
Cortisol	100 (100)
Desoxycorticosterone	0
Corticosterone	1.4 (61)
Progesterone	5.5 (20)
Compound S	10 (89)
11-OH-progesterone	0.4
18-OH-corticosterone	0
18-OH-desoxycorticosterone	0.42

In brackets the cross reaction figures of MURPHY[6] obtained with human plasma.

A typical calibration curve is given in Figure 1. The sensitivity in that case is about 25 *pg*, the standard curve being linear for values above that threshold.

The percentage of cross reaction of cortisol antisera with several steroids is given in Table I. The degree of the cross reaction was calculated according to the method of

Table II.

Extraction

0.05–0.1 ml Plasma + ca. 1000 cpm 1-2-H^3-cortisol + 0.5–0.7 ml aethanol

↓ centrifugation

↓

0.1 ml of supernatant + 1.9 ml water

↓ ↓

0.1 ml for radioimmunoassay — 0.5–1.0 ml for measuring of radioactivity (recovery)

Radioimmunoassay

0.1 ml 1-2-H^3-cortisol solution (3–5000 cpm) (0.05 *M* borate buffer with 0.1% lysozym)

0.1 ml solution with unknown amounts of cortisol (5% aethanol solution)

0.5 ml solution with cortisol antibodies (0.05 *M* borate buffer with 0.1% lysozym)

↓ incubation, 4°C, 2 h

↓ Separation of the protein-bound and -unbound radioactivity with dextran coated charcoal[a]

↓ Measuring of the protein-bound radioactivity

[a] 1 vol. activated charcoal solution (5 g Norit A, Serva, Heidelberg, in 80 ml pH 8.0, 0.05 *M* borate buffer containing 0.6% human γ-globulin) + 6 vol. dextran solution (100 mg dextran T 70, Pharmacia, Uppsala) dissolved in 80 ml γ-globulin buffer solution. Stirring for 10 min prior to use. The used volume is 0.1 ml.

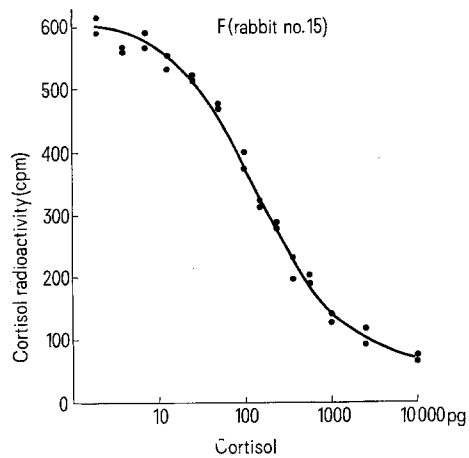

Fig. 1. Calibration curve, obtained with different amounts of unlabelled cortisol. Time of incubation: 2 h at 2°C. Ordinate: bound cortisol radioactivity (cpm). Abscissa: different amounts of cortisol.

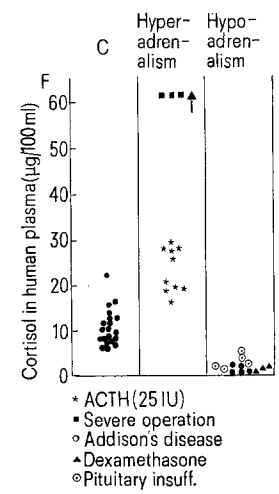

Fig. 2. Concentration of cortisol (F) in human plasma. C, control persons; ACTH, 1 or 2 h after 25 IU, i.v.; dexamethasone, 10 h after 2–4 mg dexamethasone p.o.

ABRAHAM[5]. For comparison, cross-reaction data obtained with the transcortin-binding assay[6] are included. The specificity of the antiserum, as shown by the cross reactions, is dependent on the type of transport protein used in the incubation medium. With gelatine as transport protein the specificity was highest, but since the quality of gelatine varied, preference was given to lysozyme.

The flow-sheet (Table II) indicates the various steps of the radioimmunoassay of plasma cortisol.

With the extraction of water the blank values were virtually zero. An incubation period of more than 2 hours did not give any better results. The overall recovery of tritiated cortisol added to the plasma samples prior to the extraction was 60–70%. The recovery of known amounts of cortisol added to water was 94.4 $\pm$ 2.6% ($n = 34$). The incomplete recovery might have been caused by transformation of cortisol into a less immunoactive compound. Differences in simultaneous determinations of the same samples within one assay were 12.4 $\pm$ 1.4% ($n = 37$). Day-to-day differences were 16 $\pm$ 1.9% ($n = 36$).

Radioimmunological determinations of plasma-cortisol concentrations in man gave results similar to those obtained with other methods (Figure 2). FRASER and JAMES[7] found 3.1–20.2 µg/100 ml, and SPARK[3] measured 4–24 µg/100 ml. In patients with Addison's disease and in subjects who had received dexamethasone the values were very low or undetectable.

Zusammenfassung. Durch Immunisierung von Kaninchen mit Cortisol-21-Hemisuccinat-Rinderserumalbumin-Komplex liessen sich spezifische Antikörper mit hohem Titer gewinnen. Eine radioimmunologische Bestimmungsmethode für Plasmacortisol wird angegeben. Die Methode ist besonders bei denjenigen Fällen anzuwenden, bei denen erhöhte Konzentrationen von Corticosteron oder Substanz S den «protein-binding assay» von Cortisol im Plasma stören.

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Inhibition of Testosterone Biosynthesis by Medrogestone

Medrogestone, viz. 6,17-dimethylpregn-4,6-diene-3,20-dione[1] (Colprone®) was shown to be progestational and antiandrogenic in rats[2]. The inhibitory effect of medrogestone on gonadal hormone synthesis in vitro and testosterone biosynthesis in vivo is reported herewith[3].

In vitro, with a human placental 10,000 $\times g$ supernatant fraction[4], medrogestone (1×10^{-5} M, final conc.) inhibited the conversion of dehydroepiandrosterone (DHA) to 17β-estradiol by 91%; while DHA and Δ^5-androstenediol accumulated, no testosterone was detected (Table I). The degree of inhibition was concentration dependent. In other experiments, medrogestone (1×10^{-5} M) reduced the conversion of Δ^5-androstenediol (87% inhibition) but not of testosterone or Δ^4-androstenedione to 17β-estradiol.

The effect of medrogestone on the conversion of pregnenolone to progesterone was studied with a rat ovarian 10,000 $\times g$ supernatant fraction[5]. Progesterone was isolated by thin-layer chromatography and quantified from its UV-absorption. At 1×10^{-6} M, medrogestone inhibited the formation of progesterone by 70%.

Subsequently, we investigated the effect of medrogestone on rat testes by using a 10,000 $\times g$ supernatant fraction of testicular homogenates. The reaction mixture was separated by thin-layer chromatography and the constituents were quantified by colorimetry[6]. At 1×10^{-6} M, with pregnenolone as substrate, medrogestone suppressed the formation of progesterone (90% inh.), 17-hydroxyprogesterone (84% inh.), Δ^4-androstenedione (41% inh.) and testosterone (30% inh.), respectively. Similarly, medrogestone inhibited the conversion of 17-hydroxypregnenolone to 17-hydroxyprogesterone (83% inh.), Δ^4-androstenedione (56% inh.) and testosterone (40%). Based on these results we concluded that medrogestone inhibits the 3β-hydroxysteroid dehydrogenase-$\Delta^{5,4}$-isomerase reactions.

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Table I. Effect of medrogestone on the conversion of dehydroepiandrosterone (DHA) to 17β-estradiol in vitro

	Products (µg)[a]			
	DHA[b]	Δ^5-Androstenediol-17β	Testosterone	17β-Estradiol
Control	1	1	4	22
Medrogestone ($1 \times 10^{-5} M$)	6	24	0	2

[a] Average of duplicate incubations. [b] 32 µg used per incubation.