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.

Table II. Effect of medrogestone on accessory sex organs in immature male rats treated with HCG

	Ventral prostate (mg)	Seminal vesicles (mg)
Control[a]	53 ± 4	18 ± 2
Medrogestone[b]	39 ± 2[d]	19 ± 1
HCG[c]	97 ± 5[d]	44 ± 2[d]
HCG plus Medrogestone	69 ± 3[e]	26 ± 2[e]

[a] 10 albino rats/group (Charles River), final body weight, 75 ± 1 g. [b] 100 mg/kg/day given s.c. for 4 days. [c] 2 IU (Ayerst A.P.L.) given s.c. for 4 days. [d] $p < 0.01$, compared to control group. [e] $p < 0.01$, compared to HCG-group.

Table III. Effect of medrogestone on testicular testosterone synthesis in hypophysectomized rats treated with HCG

	Testosterone (ng/g)
Control[a]	3 ± 2
Medrogestone[b]	5 ± 2
HCG[c]	202 ± 54
HCG plus Medrogestone	36 ± 6[d]

[a] 8 albino rats/group (Charles River), final body weight, 177 ± 2 g, maintained on 1% dextrose + 0.9% NaCl in the drinking water. [b] 20 mg/kg/day given s.c. for 3 days. [c] 50 IU (Ayerst A.P.L.) given s.c. on 3rd day, 24 h before sacrifice. [d] $p < 0.01$, compared to HCG-group.

In vivo, in rats (final body weight, 162 ± 2 g) receiving for 7 days a daily dose of 100 mg/kg s.c., medrogestone decreased the weights of the ventral prostate (control, 56 ± 5 mg; treated, 30 ± 3 mg; $p < 0.01$) and seminal vesicles (control, 88 ± 10 mg; treated, 36 ± 2 mg; $p < 0.01$). Similarly, in immature male rats, medrogestone significantly reduced the HCG-induced increase of the ventral prostate and seminal vesicles (Table II). Subsequently, the effect of medrogestone on the testicular synthesis of testosterone was studied in hypophysectomized rats (final body weight, 174 ± 3 g) given on the second day a subcutaneous dose of 50 IU of HCG. Testosterone was isolated by thin-layer chromatography and measured fluorometrically[7]. A dose-dependent reduction in testosterone content was observed: 3% at 5 mg/kg, 49% at 20 mg/kg and 82% at 100 mg/kg of medrogestone given daily for two days ($p < 0.01$ when compared to rats treated with HCG). After 3 days of treatment with 20 mg/kg of medrogestone, the HCG-induced increase in testicular testosterone content was decreased by 84% (Table III).

The dependence of a hyperplastic prostate on functioning testicular tissue has been established earlier[8,9] and it has been reported that some progestogens capable of lowering plasma testosterone levels produced clinical improvement in benign prostatic hyperplasia[10,11]. Our experimental findings are correlative to the recent clinical finding that medrogestone reduced the severity of benign prostatic hyperplasia in man[12].

Résumé. In vitro, la medrogestone bloque la synthèse des hormones dans les gonades par inhibition de la 3β-hydroxystéroide déshydrogénase-$\Delta^{4,5}$-isomérase. In vivo, la medrogestone réduit le niveau de testostérone dans le testicules de rat.

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Phosphorylase Activity in the Normal Human Testis: A Histochemical Study

The first histochemical demonstration of phosphorylase was carried out by TACHEUKI and KURIAKI[1]. The technique has since been modified[2–6] thus facilitating histochemical differentiation of active (a) and inactive (b) phosphorylases. In a previous study[7], using the original technique of TACHEUKI and KURIAKI, we demonstrated the presence of phosphorylase in the normal human testis. The aim of the present research was to study the type of phosphorylase present in normal human testis.

Material and methods. Biopsy specimens from testes of normal subjects between 18–35 years of age were employed. Specimens were immediately frozen at –70 °C on dry ice and absolute ethyl alcohol. Sections 12 μm thick were cut in the cryostat and mounted on glass slides. The incubation medium for phosphorylase (a) contained glucose-1-phosphate, acetate buffer pH 5.7, EDTA and NaF according to MEIJER[6]. For the study of (b) phosphorylase 20 mg of AMP was added to the incubation medium. Sections were incubated at 37 °C for 2 h, washed in 40% ethyl alcohol and fixed in absolute ethyl alcohol. The Schiff-dimedone reaction was used to stain the slices according to BULMER[8]. Control specimens were incubated in a medium without substrate.

Results. Slices incubated in the medium without AMP showed a strongly positive reaction with fine irregular granules in the seminiferous tubules. The distribution of

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