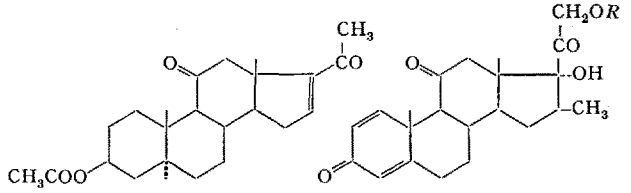
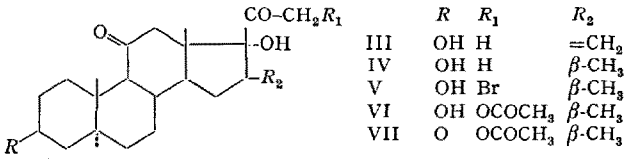
H 9.45; found: C 73.15; H 9.40. Under suitable experimental conditions (neutral pH, slow hydrogenation rate) the formation of the 16α-methyl-isomer may be completely avoided. A chloroform suspension of IV, in the presence of a small amount of methanol, was treated with bromine and hydrobromic acid to give *21-bromo-16β-methyl-5α-pregnan-3β,17α-diol-11,20-dione* (V), m.p. 220 to 223°, $[\alpha]_D$ + 90.1° (dioxane); *Anal.* calc. for $C_{22}H_{33}BrO_4$: C 59.86; H 7.54; Br 18.1; found: C 59.70; H 7.43; Br 18.38. Potassium acetate in refluxing acetone converted V to *16β-methyl-5α-pregnan-3β,17α,21-triol-11,20-dione 21-acetate* (VI), m.p. 223–227°, $[\alpha]_D$ + 95.8° (dioxane); *Anal.* calc. for $C_{24}H_{36}O_6$: C 68.58; H 8.63; found: C 68.06; H 8.54. Chromic acid in acetone[9] or chromic anhydride in acetic acid or N-bromoacetamide oxidized VI to *16β-methyl-5α-pregnan-17α,21-diol-3,11,20-trione-21-acetate* (VII), m.p. 210–213°, $[\alpha]_D$ + 120° (dioxane); *Anal.* calc. for $C_{24}H_{34}O_6$: C 68.9; H 8.19; found: C 68.3; H 7.82. Compound VII, when treated with bromine in dioxane, yielded the 2,4-dibromo-derivative (VIII), which, as crude product, was dehydrobrominated in dimethylformamide solution[10] to give *16β-methylprednisone-21-acetate* (*IX*), m.p. 224–229°, $[\alpha]_D$ + 210° (dioxane); λ_{max} 238 mμ, $E^{1\%}_{1\,cm}$ 358 (methanol). IX was de-acetylated by conventional methods to *16β-methylprednisone* (*X*), m.p. 200–205°. $[\alpha]_D$ + 200° (dioxane), $E^{1\%}_{1\,cm}$ 416 at 239 mμ in methanol. IX and X are identical with authentic specimens.



I

IX $R = COCH_3$
X $R = H$



	R	R_1	R_2
III	OH	H	$=CH_2$
IV	OH	H	β-CH_3
V	OH	Br	β-CH_3
VI	OH	$OCOCH_3$	β-CH_3
VII	O	$OCOCH_3$	β-CH_3

Zusammenfassung. Es wird über die Herstellung des 16-Methylprednisons berichtet, welche vom Hecogenin ausgehend über ca. 15 Stufen durchgeführt wird. Als wichtige Zwischenstufe treten 5α-Pregn-16-en-3β-ol-11, 20-dion-Acetat und 16-Methylen-5α-pregnan-3β,17α-diol-11,20-dion auf: letzteres wird durch eine stereospezifische katalytische Reduktion in das entsprechende 16β-Methylderivat umgewandelt.

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Research Laboratories of Lepetit S.p.A., Milano (Italy), June 26, 1961.

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Conversion of 17α-Hydroxypregnenolone to Cortisol

In the commonly accepted scheme of adrenal corticoid biosynthesis, cortisol is considered to arise from the conversion of pregnenolone (3β-hydroxy-pregn-5-en-20-one) to progesterone, which is then successively hydroxylated to cortisol. It has been demonstrated[1], however, that pregnenolone can undergo initial 17α-hydroxylation, resulting in the formation of 17α-hydroxypregnenolone, and this product has been isolated from dog adrenal vein blood[2]. It has been proposed[3] that 17α-hydroxypregnenolone is the precursor of dehydroepiandrosterone (DHA), either in the adrenal or elsewhere, and this conversion has been shown to occur in a patient with adrenal cancer[4] and in particulate fractions of beef adrenal and testis[1]. We have examined the metabolism of 17α-hydroxypregnenolone and have found that it is converted to 17α-hydroxyprogesterone, 11-deoxycortisol and cortisol by adrenal slices from the human, guinea pig and rat.

Experimental. 17α-hydroxypregnenolone-7-H^3 (New England Nuclear Corp.) was diluted with stable 17α-hydroxypregnenolone and chromatographed in the system toluene/propylene glycol. Material from the major peak contained 93% of the radioactivity and had a specific activity of 2.9×10^8 cpm/mg. Pregnenolone-7-H^3 (New England Nuclear Corp.) was diluted with stable pregnenolone and chromatographed in the system Bush B_3. The major peak contained 89% of the radioactivity and had a specific activity of 1.8×10^8 cpm/mg. Tritium and C^{14} counting were performed in the Packard Tri-Carb Scintillation spectrometer at an approximate efficiency of 15% for tritium and 80% for carbon[14].

Incubations were performed in pH 7.4 saline-phosphate buffer[5] for 3 h under air at 37°C. The human adrenals were obtained from two women undergoing adrenalectomy for breast cancer. The rats and guinea pigs were killed by decapitation.

Human adrenal slices with total weights of 3.1 g and 5.5 g were incubated with 5.2×10^6 cpm and 1.3×10^7 cpm respectively of 17α-hydroxypregnenolone. Similarly 3.1 g of slices were incubated with 2.8×10^6 cpm of pregnenolone. A total of 504 mg of guinea pig adrenal slices and 374 mg of rat adrenal slices were each incubated with 5.2×10^6 cpm of 17α-hydroxypregnenolone. After incubation, 200 γ of the following steroids were added: cortisol, 11-deoxycortisol, 17α-hydroxyprogesterone, progesterone, corticosterone and DHA. The contents of the flask were extracted with 80% acetone, defatted in cold 70% methanol, and partitioned between hexane and 70% methanol. The 3β-hydroxysteroids were separated with digitonin. The non-digitonin-precipitable steroids were initially chromatographed in chloroform/formamide. Position of the compounds was determined by UV absorption and the Zimmerman reaction, and radioactivity by scanning on a Baird-Atomic strip scanner. Evidence

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for the conversion of the labelled 17α-hydroxypregnenolone to other steroids was obtained as follows:

Cortisol was eluted and chromatographed in system Bush C. An aliquot was acetylated with acetic anhydride in pyridine and chromatographed in Bush B_3 and cyclohexane:dioxane/methanol:water (4:4/2:1)[6]. An aliquot of the cortisol from Bush C was oxidized with sodium bismuthate and chromatographed in Bush B_1. After elution, it was oxidized with chromic acid for 10 min and rechromatographed in system Bush B_1, in which the Rf was equal to that of androst-4-en-3,11,17-trione. The radioactive peak migrated with the carrier steroid throughout. In one study with the human adrenal slice, the cortisol was acetylated with C^{14}-acetic anhydride and the ratio of H^3 to C^{14} determined after chromatography in Bush B_3, cyclohexane:dioxane/methanol:water, and after elution with 2% acetone in chloroform from a silica gel column. The specific activities of the eluted cortisol acetate were 93, 90, and 88 in the two paper systems and from the peak tubes of the column respectively.

17α-hydroxyprogesterone was chromatographed in ligroin/propylene glycol and Bush B_3. It could not be acetylated with acetic anhydride in pyridine. Acetylation in about 60% yield was accomplished by adding *p*-toluenesulfonic acid to the acetylation reagents. The resulting compound was chromatographed in Bush A where it migrated at the same rate as 17α-acetoxyprogesterone. The radioactive peak coincided with the carrier steroid throughout.

11-deoxycortisol was chromatographed in Bush B_3, an aliquot acetylated and rechromatographed in Bush A and the dioxane system. The acetylated product was reduced with sodium borohydride, oxidized with sodium bismuthate and chromatographed in Bush A. An aliquot of the material from Bush B_3 was oxidized with bismuthate. These two bismuthate oxidation products had an Rf in Bush A identical to androstenedione. The radioactive peak migrated with the carrier compound in all systems.

Dehydroepiandrosterone was successively chromatographed in ligroin/propylene glycol and Bush A. After acetylation, the product was chromatographed in heptane/phenyl Cellosolve. The mobilities of the radioactive peak and the carrier compound coincided. In the human and guinea pig adrenal slices, the DHA contained less than 5% of the combined radioactivity of the three 17α-hydroxysteroids discussed above. Tritium-labelled DHA could not be identified in the rat adrenal slice incubation.

Corticosterone and *progesterone* were not tritium-labelled after incubation with 17α-hydroxypregnenolone.

The human adrenal slices converted 9% of the labelled 17α-hydroxypregnenolone to cortisol and 6% of the pregnenolone to cortisol. Thus 17α-hydroxypregnenolone was at least as good a precursor of cortisol as was pregnenolone. In several separate but not paired incubations with guinea pig adrenal slices, pregnenolone and 17α-hydroxypregnenolone were equally active as cortisol precursors.

Discussion. The existence of a biosynthetic pathway to 17α-hydroxyprogesterone via 17α-hydroxypregnenolone has been tacitly assumed[1], but evidence has not been presented to support this. It is clear from the present study that 17α-hydroxypregnenolone is a good substrate for the adrenal 3β-ol dehydrogenase system and that in the adrenal slice this constitutes a major metabolic transformation of the compound. The preliminary comparison of the relative activities of pregnenolone and 17α-hydroxypregnenolone suggests that 17α-hydroxylation of pregnenolone may be as rapid as the conversion of pregnenolone to progesterone. These results do not support the view that 17α-hydroxypregnenolone is the important precursor of DHA *within the adrenal gland*, since the conversion of 17α-hydroxypregnenolone to DHA was relatively small. This, of course, does not exclude the conversion of 17α-hydroxypregnenolone to DHA by desmolases outside the adrenal gland as has been suggested earlier[3]. Indeed, the finding that 17α-hydroxypregnenolone was a precursor of urinary pregnanetriol in two elderly adrenalectomized women[7] suggests that an extra-adrenal 3β-ol dehydrogenase system may be present.

It is of interest that 17α-hydroxypregnenolone was a suitable substrate for the 3β-ol-dehydrogenase system of the rat adrenal. Thus, when a 17α-hydroxylated substrate was present, the rat adrenal was able to synthesize cortisol[8].

Zusammenfassung. Nebennierenschnitte von Ratten, Meerschweinchen und Menschen synthetisierten 17α-Hydroxyprogesteron, 11-Deoxycortisol und Cortisol aus 17α-Hydroxypregnenolon. Diese Verbindung, als Ausgangsprodukt für die Cortisolsynthese, ist ebenso wirksam wie pregnenolon. Die Umwandlung von 17α-Hydroxypregnenolon in Dehydroepiandrosteron verläuft weniger vollkommen als zu 17α-Hydroxycorticoiden.

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Identification du glucosiduronate de 5β-pregnane 3α,21-diol 20-one (THDOC) après administration de cortexone

Nous avons administré à un homme normal 50 mg de cortexone par voie orale en 3 prises et les urines de 24 h ont été recueillies. Les stéroïdes libres et conjugués sont extraits à pH 7 par le mélange éther/éthanol 3/1 v/v[1]. Les corticostéroïdes libres sont extraits par le dichlorométhane et les corticostéroïdes conjugués sont fractionnés sur alumine en ester-sulfates et glucosiduronates[2].

La fraction glucosiduronate a été chromatographiée sur celite purifié en utilisant les solvants: toluène-acétate de buthyle-butanol (40:50:10) comme phase mobile et le mélange acide acétique-eau-méthanol (5:45:50)[3] comme phase stationnaire. Les glucosiduronates sont détectés par la réaction de Tollens, rassemblés et chromatograhpiés sur papier dans ce même système. Les taches de glucosiduronates ont été révélées par le réactif acide acétique-sulfurique 9/1 (v+v)[4].

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