The metabolism of the naturally occurring corticosteroids has been investigated extensively in systems both in vivo and in vitro (1, 2). In contrast, little information is available concerning the metabolic pathways of the 1-dehydrocorticosteroids. It has been demonstrated that in men the major metabolic reactions of prednisolone and prednisone involve reduction-oxidation reactions at C-11 and reduction at C-20 (3-7). In addition, partial or complete hydrogenation of the cross-conjugated system in ring A (6) and cleavage of the dihydroxyacetone side chain have been reported (6, 7). The partial or complete disappearance of the cross-conjugated system in ring A (8, 9) and the loss of the α-ketolic function in the side chain (9) have been demonstrated in vitro with rat liver preparations.

We have investigated the metabolism of prednisolone in vitro with a rat liver preparation. Certain properties of the enzymatic systems involved in the transformations noted and the structures of three metabolites isolated are reported in this communication.

METHODS

Material—Albino rats weighing 200 to 250 gm. were decapitated and the livers were quickly removed and chilled in ice. The livers were homogenized in a Porter-Elvehjem homogenizer with 4.5 volumes of Krebs' phosphosaline buffer (pH 7.4) containing 248 mg. of Versene per liter of solution. The homogenate was strained through gauze and centrifuged for 15 minutes at 6000 x g. The supernatant fluid was diluted with an equal volume of a saturated ammonium sulfate solution, the pH adjusted to 7.0, and the fluid again centrifuged for 15 minutes at 6000 x g. The resulting supernatant fluid was used in most of the incubations.

Incubations—In small scale experiments 300 μg. of prednisolone were incubated for 1 hour at 37° in an atmosphere of air with 4.5 ml. of the enzyme preparation. The cofactor requirements of the enzyme preparation were studied. For isolation of metabolites 5 mg. of prednisolone were incubated for 2 hours at 37° with 0.0 ml. of the enzyme preparation, to which 0 ml. of 0.01 m nicotinamide, 6 ml. of 0.01 m sodium citrate, and 500 mg. of DPNH were added.

Extraction—At the end of the incubation, sufficient acetone was added to the flasks to make a final concentration of 70 per cent acetone in the mixture. The flasks were left overnight in a refrigerator, then filtered and the residue washed with hot acetone. The volume of the filtrate was reduced in vacuo to 0.5 of the original, the aqueous phase was shaken three times with 0.3-volume portions of petroleum ether, and the petroleum ether extracts were discarded. The aqueous phase was then distilled in vacuo to a small volume and the steroids were recovered with ethyl acetate. The combined ethyl acetate extracts were dried over sodium sulfate and concentrated to a residue, which was then dissolved in chloroform and chromatographed on Florisil (10). The steroids were eluted with a mixture of 25 per cent methanol in chloroform.

Analytical Procedures—Aliquots were removed for the estimation of changes in the side chain and in ring A. The disappearance of the dihydroxyacetone group was measured by the Porter-Silber method (11) and the reduction of the cross-conjugated system in ring A by the decrease in optical density at 240 mμ. Corrections for blank experiments were made. The nature of the metabolites formed was examined by paper chromatography in the chloroform formamide system (12, 19). For the isolation of the transformation products the incubation extracts from two experiments were combined (see above) and chromatographed on paper. Several zones were rechromatographed until single spots were obtained. The appropriate areas were then eluted and further purification was pursued through crystallization.

Melting points were taken on a micro hot stage and were recorded as read. Spectra of solutions of metabolites in sulfuric acid were recorded in the range of 220 to 600 mμ 2 hours after the addition of the acid. The infrared spectra were obtained from material incorporated into rectangular potassium bromide prisms (13) on a Perkin-Elmer model 13C spectrometer. Ultraviolet spectra were recorded in methanol. For identification purposes the spectra of sulfuric acid solutions and the infrared spectra were respectively compared with those of authentic samples.

RESULTS

The efficiency of the extraction method employed and the chromatography on Florisil were tested on numerous occasions and recoveries of 80 to 95 per cent of the added prednisolone were obtained. Incubation of prednisolone with homogenates of rat liver in a Krebs' buffer resulted in a 36 per cent loss of the cross-conjugated system in ring A and a 10 per cent loss of the dihydroxyacetone side chain (Table I). Addition of 0.005 moles of DPNH did not influence the rate of metabolism in
ring A but increased by 20 per cent the disappearance of the side chain. No further changes occurred after the addition of nicotinamide. However, the addition of citrate ions increased appreciably both the changes in ring A and in the side chain.

Fractionation of the metabolites on paper gave a similar pattern of spots for all incubation mixtures. The spots and their characteristics are listed in Table II. Because Spot 1 was found only on chromatograms of residues of experiments to which DPNH was added and in extracts of blank experiments with added DPNH but without prednisolone, it was most likely that the product was not a steroid. Spot 2 was detected on the chromatogram of the residue of the "large" scale experiment. It seemed that the concentration of this substance in small scale experiments was insufficient for detection. All spots with the exception of Spot 8 absorbed ultraviolet light.

**Isolation of 11β,17α,20β,21-Tetrahydroxy-4-Pregnene-3-One from Spot 4**—The residue was eluted and rechromatographed in toluene propylene glycol to yield a single spot which appeared dark when observed under the ultraviolet lamp. The eluted product did not react with blue tetrazolium (14) but gave a blue coloration with an ethanolic solution of phosphomolybdic acid (15). The substance was acetylated in the usual manner and was crystallized from ethyl acetate neohexane, m.p. 205–215°C; \( \lambda_{	ext{max}}^{\text{MClO}}, 240 \text{ miz}; \epsilon, 7,000 \). The infrared spectrum and that of a sulfuric acid solution of the product were identical to those of an authentic sample of 11β,17α,20β,21-tetrahydroxy-4-pregnene-3-one.

**Isolation of Prednisolone (11β,17α,21-Trihydroxy-1,4-Pregnadiene-3,20-Dione) from Spot 6**—Rechromatography of the residue in toluene propylene glycol gave a single spot, which was eluted and twice crystallized from acetone petroleum ether, m.p. 225–228°C; \( \lambda_{\text{max}}^{\text{MClO}}, 241 \text{ miz}; \epsilon, 15,500 \). The spectrum of a sulfuric acid solution and the infrared spectrum of the metabolite were identical with those of prednisolone. The isolated product was unchanged starting material not transformed during incubation. The product did not react with blue tetrazolium (14) but gave a blue coloration with an ethanolic solution of phosphomolybdic acid (15). The substance was acetylated in the usual manner and was crystallized from ethyl acetate neohexane, m.p. 212–215°C; \( \lambda_{\text{max}}^{\text{MClO}}, 242 \text{ miz}; \epsilon, 16,900 \). The infrared spectra of the free alcohol and of the acetate were identical with those of cortisol and its acetate.

**Isolation of Cortisol from Spot 7**—After rechromatography in toluene propylene glycol the eluted material was chromatographed on silica gel. The eluates, which gave a purple coloration with blue tetrazolium reagent, were combined and crystallized from ethyl acetate neohexane, m.p. 192–195°C. Acetylation in the usual manner gave an acetate m.p. of 205–215°C; \( \lambda_{\text{max}}^{\text{MClO}}, 244 \text{ miz}; \epsilon, 15,500 \). The infrared spectra of the free alcohol and of the acetate were identical with those of cortisol and its acetate.

**Isolation of 11β,17α,21-Trihydroxyallopregnane-3,20-Dione from Spot 8**—The residue from this zone was rechromatographed in toluene propylene glycol to yield two spots, both of which reacted with blue tetrazolium. The major spot was then eluted and rechromatographed in the Bush system C (16). A single spot was detected which was eluted and twice crystallized from acetone petroleum ether, m.p. 220–224°C; \( \lambda_{\text{max}}^{\text{MClO}}, \) nonc. The infrared spectrum of the metabolite was identical to that of authentic 11β,17α,21-trihydroxyallopregnane-3,20-dione.

**DISCUSSION**

Results reported in this communication show the susceptibility of prednisolone to enzymatic attack in vitro by preparations of rat liver and are in agreement with those of other investigators (8, 9, 17). Neither DPNH nor nicotinamide additions had any influence on the reduction of the 1,4-diene-3-one group. However, the addition of citrate ions increased the rate of the hydrogenation of the cross-conjugated system. This appears to be in agreement with the observations reported that TPNH is a cofactor (8). The disappearance of the dihydroxyacetone side chain with the formation of a glycercol type group was enhanced by the addition of DPNH. The dependence of the reduction of the 20-carboxyl function on phosphopyridine nucleotide has been observed previously (18).
The isolation and identification of the metabolites reported in this communication is the first illustration of the nature of the metabolic transformations in vitro of prednisolone by preparations of rat liver. The isolation of cortisol was of special interest because it constitutes the first demonstration of the conversion of prednisolone to the naturally occurring hormones. That the reduction of the olefinic bond at C-1 occurs before that at C-4 has been indicated previously (8). This seems to be in contrast to the situation in men, where the reverse situation has been recorded, i.e. the hydrogenation of the C-4 double bond occurring prior to that at C-1 (6). Thus far the presence of cortisol has not been demonstrated in human urine after the administration of prednisolone (3-7).

The finding of cortisol suggested the occurrence of other metabolites similar to those obtained in vitro with corticoids and preparations of rat liver (19-23). The isolation of 11β,17α,21-trihydroxyallopregnane-3,20-dione and 11β,17α,20β,21-tetrahydroxy-4-pregnene-3-one was in accord with these predictions. The isolated metabolites are of additional interest because the substances are in the “intermediate” reduction stage. Incubation in vitro of corticoids with preparations of rat liver (8, 21, 22) and especially after the perfusion of cortisol or cortisone through rat livers (19, 20) has shown that the bulk of the isolated metabolites were either fully reduced to the corresponding alcohols or to the “allotetrahydro” derivatives. Based on studies on the metabolism in vitro of naturally occurring corticosteroids and the results reported in this communication, the transformation of prednisolone to other C11 and C19 compounds, both the trans and cis configuration at C-5 may be expected. The isolation of 3α,11β,17α,21-tetrahydroxyprogren-20-one, 3α,11β,17α,21-tetrahydroxyallopregn-20-one (6), 11β-hydroxy-1,4-androstadiene-3,17-dione, 1,4-androstadiene-3,11,17-trione (7), 3α,11β-dihydroxyetiocholane-17-one, 3α-hydroxyetiocholane-11,17-one (6), and 32,17β-dihydroxyetiocholane-11-one (24) from human urine after the administration of prednisolone seems to support this view. The inability to detect other products was probably caused by their low concentration in the metabolic residue derived from the incubation of 10 mg. of prednisolone.

SUMMARY

1. Prednisolone was incubated with an ammonium sulfate treated supernatant fluid (6000 × g) from a homogenate of rat liver.
2. Certain properties of the enzymatic systems involved were investigated.
3. Three metabolites of prednisolone were isolated and identified: cortisol; 11β,17α,21-trihydroxyallopregnane-3,20-dione; and 11β,17α,20β,21-tetrahydroxy-4-pregnene-3-one. The presence of other metabolites was demonstrated.
4. The significance of the findings was briefly discussed.

REFERENCES
