

# Isolation of 15 $\alpha$ -Hydroxyprogesterone from Human Pregnancy Urine\*

G. Giannopoulos and S. Solomon

**ABSTRACT:** Two pools of urine were obtained from normal subjects in the third trimester of pregnancy. Purified [7- $^3\text{H}$ ]15 $\alpha$ -hydroxyprogesterone, prepared microbiologically, was added to the urine as a recovery marker and the steroid was isolated and identified in both urine pools. The amount of 15 $\alpha$ -hydroxyprogesterone excreted in the urine was 34  $\mu\text{g}/\text{day}$  in one

pool and 28  $\mu\text{g}/\text{day}$  in the second, measured by the isotope derivative procedure using [1- $^{14}\text{C}$ ]acetic anhydride. These findings demonstrate that 15 $\alpha$ -hydroxyprogesterone is a normal urinary excretion product in the third trimester of pregnancy. The possible physiological role of 15 $\alpha$ -hydroxyprogesterone in pregnancy is discussed.

Recently the 15 $\alpha$ -hydroxylation of steroids has been demonstrated in mammalian tissues. Knuppen and Breuer (1964) reported the *in vitro* conversion of estrone<sup>1</sup> to 15 $\alpha$ -hydroxyestrone by bovine adrenal tissue, and this report was followed by the demonstration of Schwers *et al.* (1965) that 15 $\alpha$ -hydroxyestradiol could be isolated in an aqueous soluble form from the liver of previable human fetuses perfused with [ $^3\text{H}$ ]estrone, [4- $^{14}\text{C}$ ]estradiol, and [4- $^{14}\text{C}$ ]estrone sulfate. Subsequently, 15 $\alpha$ -hydroxyestrone was isolated from human pregnancy urine by Knuppen *et al.* (1965a) while the formation of 15 $\alpha$ -hydroxyestradiol has been shown to occur in human adrenals (Knuppen *et al.*, 1965b). Recently, Knuppen *et al.* (1966) also isolated 15 $\beta$ -hydroxyestrone from human pregnancy urine. When the mid-term previable human fetus was perfused with [4- $^{14}\text{C}$ ]progesterone (Bird *et al.*, 1966), 15 $\alpha$ -hydroxyprogesterone was looked for in the adrenals and liver but was not found. Our working hypothesis

was that 15 $\alpha$ -hydroxylation of neutral steroids occurred in the human fetus later in pregnancy, and an investigation was undertaken to isolate such metabolites from late-pregnancy urine. This paper describes the isolation of 15 $\alpha$ -hydroxyprogesterone from late pregnancy urine.

## Materials and Methods

Techniques used in counting  $^{14}\text{C}$  and  $^3\text{H}$ , the preparation of solvents, paper and column chromatography, enzymatic hydrolysis of urinary steroid conjugates, and infrared analysis have been previously described (Ruse and Solomon, 1966). In these investigations,  $^3\text{H}$  and  $^{14}\text{C}$  were counted using a Model 3002 Packard liquid scintillation spectrometer. At double-label settings the efficiency for  $^3\text{H}$  was 30% and for  $^{14}\text{C}$  64%. The following solvent systems have been employed for paper and Celite partition chromatography: (A) isooctane-toluene-methanol-water (25:25:35:15), (B) *n*-heptane-ethyl acetate-methanol-water (50:50:65:35), (C) toluene-propylene glycol, and (D) Skellysolve B-methanol-water (100:90:10).

**Preparation of [7- $^3\text{H}$ ]15 $\alpha$ -Hydroxyprogesterone.** Labeled 15 $\alpha$ -hydroxyprogesterone was prepared by the incubation of [7- $^3\text{H}$ ]progesterone with a 15 $\alpha$ -hydroxylating microorganism. The microbiological hydroxylation was performed through the courtesy of Dr. P. A. Diassi, Squibb Institute for Medical Research. A total of 1 mc of [7- $^3\text{H}$ ]progesterone (New England Nuclear Corp.) was diluted with 100 mg of carrier and the mixture was fermented with *Calletotrichum linicola* in a manner similar to that described for the 16 $\alpha$ -hydroxylation of progesterone (Ruse and Solomon, 1966). Following incubation, the whole broth was extracted with chloroform and the residue obtained was crystallized from acetone-hexane to yield 27.5 mg of crystals, mp 210–220°, and a mother liquor which weighed 40 mg. Preliminary chromatographic analysis indicated that the crystals were contaminated

\* From the Departments of Biochemistry and Experimental Medicine, McGill University, and the University Clinic, Royal Victoria Hospital, Montreal, Canada. Received January 12, 1967. This work was supported by grants from the Medical Research Council of Canada (MT-1658) and the U. S. Public Health Service, Institute of Arthritis and Metabolic Diseases (AM-04329).

<sup>1</sup> The following trivial names and abbreviations are used: estrone, 3-hydroxyestra-1,3,5(10)-trien-17-one; estradiol, estra-1,3,5(10)-triene-3,17 $\beta$ -diol; 15 $\alpha$ -hydroxyestrone, 3,15 $\alpha$ -dihydroxyestra-1,3,5(10)-trien-17-one; 15 $\alpha$ -hydroxyestradiol, estra-1,3,5(10)-triene-3,15 $\alpha$ ,17 $\beta$ -triol; estrone sulfate, 17-oxoestra-1,3,5(10)-trien-3-yl sulfate; 15 $\beta$ -hydroxyestrone, 3,15 $\beta$ -dihydroxyestra-1,3,5(10)-trien-17-one; progesterone, pregn-4-ene-3,20-dione; 15 $\alpha$ -hydroxyprogesterone, 15 $\alpha$ -hydroxypregn-4-ene-3,20-dione; 16 $\alpha$ -hydroxyprogesterone, 16 $\alpha$ -hydroxypregn-4-ene-3,20-dione; 12 $\beta$ ,15 $\alpha$ -dihydroxyprogesterone, 12 $\beta$ ,15 $\alpha$ -dihydroxypregn-4-ene-3,20-dione; 11 $\alpha$ ,15 $\alpha$ -dihydroxyprogesterone, 11 $\alpha$ ,15 $\alpha$ -dihydroxypregn-4-ene-3,20-dione; deoxycorticosterone, 21-hydroxypregn-4-ene-3,20-dione; 15 $\alpha$ -hydroxytestosterone, 15 $\alpha$ ,17 $\beta$ -dihydroxyandrost-4-en-13-one; 15 $\alpha$ -hydroxydeoxycorticosterone, 15 $\alpha$ ,21-dihydroxypregn-4-ene-3,20-dione; 15 $\beta$ -hydroxydeoxycorticosterone, 15 $\beta$ ,21-dihydroxypregn-4-ene-3,20-dione; DDQ, dichlorodicyanobenzoquinone.

with small amounts (maximum 5%) of 12 $\beta$ ,15 $\alpha$ -dihydroxyprogesterone, 11 $\alpha$ ,15 $\alpha$ -dihydroxyprogesterone, and progesterone. For further purification, 650  $\mu$ g of the crystals was chromatographed on two papers in system A for 5 hr and the ultraviolet-absorbing material with the mobility of 15 $\alpha$ -hydroxyprogesterone was eluted. An aliquot of the eluted material was mixed with carrier 15 $\alpha$ -hydroxyprogesterone and the mixture was crystallized from acetone-Skellysolve B and from methanol. As is shown in Table I the specific

TABLE I: Proof of Radiochemical Purity of [7- $^3$ H]15 $\alpha$ -Hydroxyprogesterone Prepared Microbiologically.

Crystzn	Sp Act. (dpm/mg)	
	X11s <sup>a</sup>	M <sub>L</sub> <sup>a</sup>
1	6340	6430
2	6350	6370
Calcd <sup>b</sup>	6410	

<sup>a</sup> X11s = crystals; M<sub>L</sub> = mother liquors. <sup>b</sup> An aliquot of the material eluted from paper containing  $3.5 \times 10^5$  dpm was mixed with 54.1 mg of carrier 15 $\alpha$ -hydroxyprogesterone prior to crystallization. The carrier 15 $\alpha$ -hydroxyprogesterone was obtained through the courtesy of Dr. P. A. Diassi and had a melting point of 231–232°.

activities of the crystals and mother liquors were constant and agreed well with the calculated value. From these data it was calculated that the [7- $^3$ H]15 $\alpha$ -hydroxyprogesterone was at least 99% pure, and it was this material which was used in the experiments to be described.

**Standardization of [1- $^{14}$ C]Acetic Anhydride.** Two solutions of [1- $^{14}$ C]acetic anhydride, 30% (v/v) in benzene, were standardized by the acetylation of carrier deoxycorticosterone. The acetate was purified by chromatography on an alumina column and then crystallized to constant specific activity. Specific activities were determined by weighing the crystals and mother liquors on a microbalance and by taking appropriate aliquots for the determination of radioactivity. Solutions 1 and 2 had specific activities of 2840 and 116 dpm/ $\mu$ g of deoxycorticosterone acetate, respectively.

**Determination of the Specific Activity of [7- $^3$ H]15 $\alpha$ -Hydroxyprogesterone.** An aliquot of the purified [7- $^3$ H]15 $\alpha$ -hydroxyprogesterone was acetylated with [1- $^{14}$ C]acetic anhydride (solution 1) and the acetylated product was mixed with carrier 15 $\alpha$ -acetoxyprogesterone. This mixture was purified on a small alumina column, and elution with benzene-Skellysolve B (4:1) afforded a residue which was crystallized from methanol-ether, acetone-Skellysolve B, and methanol-ether. The specific activities and the  $^3$ H: $^{14}$ C ratios

of the crystals and mother liquors were determined and are shown in Table II. Constant specific activities and  $^3$ H: $^{14}$ C ratios were obtained in the crystals and mother liquors. From the final  $^3$ H: $^{14}$ C ratio and the specific activity of the [1- $^{14}$ C]acetic anhydride, the specific activity of [7- $^3$ H]15 $\alpha$ -hydroxyprogesterone was calculated to be  $1.74 \times 10^4$  dpm/ $\mu$ g.

**Derivative Formation.** A derivative of 15 $\alpha$ -acetoxyprogesterone was prepared by reduction of the 20-ketone with NaBH<sub>4</sub> as described by Norymberski and Woods (1955), followed by oxidation of the allylic alcohol at C-3 with dichlorodicyanobenzoquinone (DDQ) as described by Burn *et al.* (1960). The residue obtained after oxidation with the DDQ reagent was chromatographed on a small silica gel column, and elution with 3% ethanol in benzene yielded a yellow oil. This oil was crystallized from methanol-ether-Skellysolve B to yield small coarse needles, mp 175–177°. An infrared spectrum (KBr) of the crystalline derivative indicated an absence of the absorption band in the position of the 20-ketone and a retention of the bands characteristic of the acetate and the  $\alpha,\beta$ -unsaturated ketone. We were not able to compare this derivative to an authentic compound, but the structure of 15 $\alpha$ -acetoxy-20 $\beta$ -hydroxypregn-4-en-20-one may be assigned to it with some confidence.

## Experimental Section and Results

**Experiment 1.** A 5-day urine pool was collected from normal pregnant women in the 28th to 36th week of gestation. The urinary steroid conjugates were hydrolyzed with Glusulase (Endo Laboratories, Garden City, N. Y.), 5 ml/l. of urine at 37° for 5 days, and a neutral extract was prepared which weighed 1.6 g. To this extract was added  $1.74 \times 10^8$  dpm of [7- $^3$ H]15 $\alpha$ -

TABLE II: Determination of the Specific Activity of [7- $^3$ H]15 $\alpha$ -Hydroxyprogesterone.

Crystzn	Specific Activity (dpm/mg)			
	X11s <sup>a</sup>		M <sub>L</sub> <sup>a</sup>	
	$^3$ H	$^3$ H: $^{14}$ C	$^3$ H	$^3$ H: $^{14}$ C
1	14,500	5.32	15,000	1.62
2	14,800	5.41	14,500	4.92
3	14,800	5.45	14,800	5.39
Calcd <sup>b</sup>	14,500			

<sup>a</sup> See footnote a of Table I. <sup>b</sup> An aliquot of [7- $^3$ H]-15 $\alpha$ -hydroxyprogesterone containing  $7.10 \times 10^5$  dpm was acetylated with [1- $^{14}$ C]acetic anhydride (solution 1) and the product was mixed with 52.9 mg of carrier 15 $\alpha$ -acetoxyprogesterone. The mixture was purified by chromatography on an alumina column prior to crystallization and the calculated specific activity is based on the  $^3$ H disintegrations per minute and weight eluted from the column.

TABLE III: Purity of 15 $\alpha$ -Hydroxyprogesterone Isolated from Urine in Expt 1.

Crystzn	Specific Activity (dpm of $^3\text{H}/\text{mg}$ )							
	15 $\alpha$ -Acetoxypregsterone <sup>a</sup>				15 $\alpha$ -Acetox-20 $\beta$ -hydroxypregn-4-en-3-one <sup>b</sup>			
	X11s <sup>c</sup>	$^3\text{H}:^{14}\text{C}$	M <sub>L</sub> <sup>c</sup>	$^3\text{H}:^{14}\text{C}$	X11s	$^3\text{H}:^{14}\text{C}$	M <sub>L</sub>	$^3\text{H}:^{14}\text{C}$
1	13,500	2.0	13,200	0.9	13,600	2.0	13,800	2.0
2	13,500	2.0	13,300	1.8	13,400	2.0	13,500	2.0
3	13,500	2.0	13,400	2.0	13,500			
Calcd	12,400				13,500			

<sup>a</sup> A total of  $6.5 \times 10^5$  dpm and 53.8 mg of the acetate was eluted from the alumina column and the calculated specific activity was based on these values. <sup>b</sup> After the third crystallization 44 mg of the acetate was reduced with  $\text{NaBH}_4$  and the allylic alcohol at C-3 was oxidized with the DDQ reagent. <sup>c</sup> See footnote *a* of Table I.

hydroxyprogesterone having a specific activity of  $1.74 \times 10^4$  dpm/ $\mu\text{g}$ , and it was then chromatographed on a 160-g silica gel column which was developed with methylene chloride and then with increasing concentrations of ethanol in methylene chloride. The effluent was collected in 100-ml fractions at a rate of one fraction per hour. A single radioactive band was eluted with 3% ethanol in methylene chloride (fractions 40–48) to yield a residue which weighed 84 mg and contained  $1.68 \times 10^6$  dpm. It was then chromatographed on a 100-g Celite column using system B. The residue eluted in the sixth to eighth holdback volumes weighed 11.8 mg and contained  $1.55 \times 10^6$  dpm. It was chromatographed on two papers in system C for 10 hr and the material corresponding in polarity to 15 $\alpha$ -hydroxyprogesterone was eluted to yield a residue which weighed 1.45 mg and contained  $1.35 \times 10^6$  dpm. Crystallization of this material from acetone–Skellysolve B yielded 0.44 mg of small plates (mp 218–221°) whose infrared spectrum (KBr) was identical with that of 16 $\alpha$ -hydroxyprogesterone. The crystals contained  $1.0 \times 10^5$  dpm, and the major portion of radioactivity was found in the mother liquors ( $1.1 \times 10^6$  dpm). After a large number of trial chromatograms using thin layer, paper, and Celite column systems it was found that prolonged chromatography on paper in system D would separate 15 $\alpha$ - from 16 $\alpha$ -hydroxyprogesterone. As a result the remaining mother liquors ( $9.9 \times 10^5$  dpm in 0.54 mg) were chromatographed on paper in system D for 5 days. Two ultraviolet-positive bands were resolved on the paper at an average distance of 9.5 and 12.5 cm, corresponding in mobility to 15 $\alpha$ - and 16 $\alpha$ -hydroxyprogesterone, respectively. Scanning of the paper revealed that the radioactivity present coincided with the ultraviolet band having the polarity of 15 $\alpha$ -hydroxyprogesterone. This material was eluted from the paper and the residue obtained was chromatographed on a 1-g alumina column. Elution with 1.5% ethanol in benzene yielded a white residue which contained  $9.5 \times 10^5$  dpm and weighed 0.2 mg. The infrared spectrum (KBr) of this material was almost identical

with that of authentic 15 $\alpha$ -hydroxyprogesterone; the only difference noted was an extra band at  $1735\text{ cm}^{-1}$ .

The remainder contained  $7.7 \times 10^5$  dpm, and it was acetylated with  $[1\text{-}^{14}\text{C}]\text{acetic anhydride}$  (solution 1). This product was mixed with 52.2 mg of carrier 15 $\alpha$ -acetoxypregsterone and the mixture was chromatographed on a 5-g alumina column. Elution with benzene–Skellysolve B (4:1) yielded a residue which weighed 53.8 mg and contained  $6.5 \times 10^5$  dpm of  $^3\text{H}$ . It was crystallized from methanol–ether, acetone–Skellysolve B, and ether, and the specific activities as well as the  $^3\text{H}:^{14}\text{C}$  ratios were determined on the crystals and mother liquors, as shown in Table III. Constant specific activity was achieved in the crystals and in the mother liquors. The third crystals, as well as the second and third mother liquors, were combined to give 44 mg of material. It was dissolved in 11 ml of methanol and 9.0 mg of  $\text{NaBH}_4$  was added to the solution. When the reaction was stopped, the product was extracted with ethyl acetate. After evaporation of the solvent the residue obtained was dissolved in 3 ml of dioxane to which was added 32.6 mg of DDQ reagent. Following oxidation the product formed was purified by chromatography on a 5-g alumina column, and elution with benzene yielded 30.9 mg of crystalline material containing  $4.1 \times 10^5$  dpm of  $^3\text{H}$ . This derivative was crystallized from ether–methanol and from methanol–ether–Skellysolve B and the specific activities of the crystals and mother liquors were determined and found to be constant as is shown in Table III. Using the final  $^3\text{H}:^{14}\text{C}$  ratio and the specific activity of the acetic anhydride, the specific activity of the urinary 15 $\alpha$ -hydroxyprogesterone was calculated to be  $6.4 \times 10^3$  dpm/ $\mu\text{g}$  of  $^3\text{H}$ . From this final specific activity it was calculated that the amount of 15 $\alpha$ -hydroxyprogesterone excreted in the urine was 34  $\mu\text{g}/\text{day}$ .

**Experiment 2.** In order to confirm the isolation of 15 $\alpha$ -hydroxyprogesterone a second experiment was performed using a larger pool of urine. A 14-day urine collection was obtained from a normal subject in the

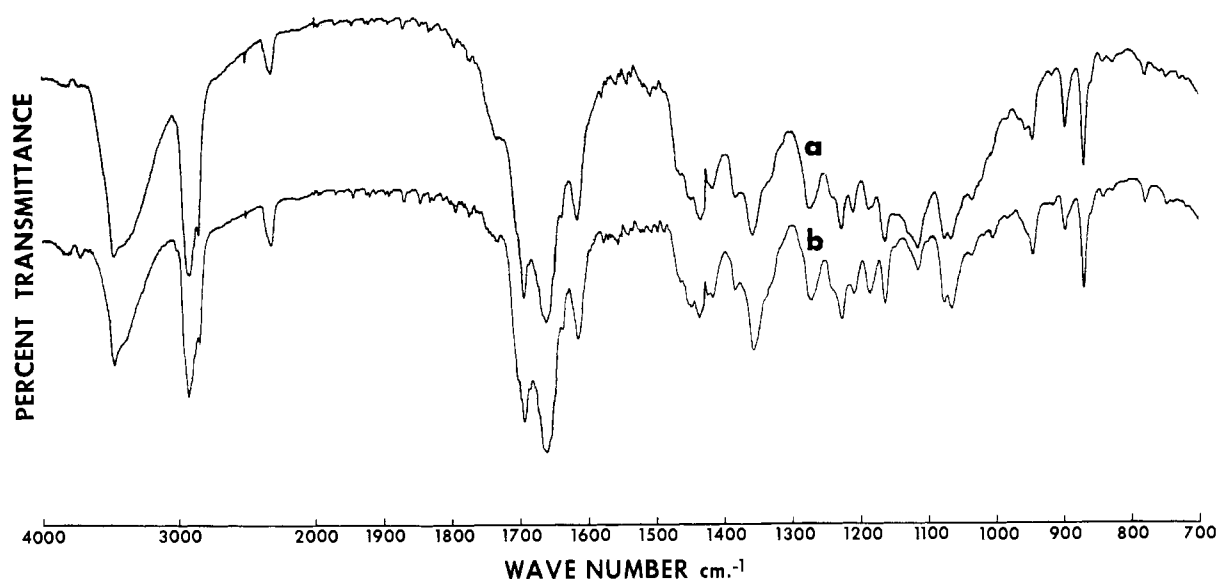


FIGURE 1: Infrared spectra of (a) the urinary metabolite and (b) authentic  $15\alpha$ -hydroxyprogesterone. KBr disks were used and the spectrum obtained with  $37\ \mu\text{g}$  of the crystalline urinary metabolite was compared to that obtained with  $60\ \mu\text{g}$  of the standard.

TABLE IV: Purity of  $15\alpha$ -Hydroxyprogesterone Isolated from Urine in Expt 2.

Crystzn	Specific Activity (dpm of $^3\text{H}/\text{mg}$ )							
	$15\alpha$ -Acetoxyprogesterone <sup>a</sup>				$15\alpha$ -Acetoxy- $20\beta$ -hydroxypregn-4-en-3-one <sup>b</sup>			
	X11s <sup>c</sup>	$^3\text{H};^{14}\text{C}$	$\text{M}_\text{L}$ <sup>c</sup>	$^3\text{H};^{14}\text{C}$	X11s	$^3\text{H};^{14}\text{C}$	$\text{M}_\text{L}$	$^3\text{H};^{14}\text{C}$
1	2750	11.5	2760	9.7	2720	11.2	2720	11.3
2	2740	11.3	2740	11.1	2740	11.2	2720	11.4
3	2740	11.2	2720	11.4				
Calcd	2760				2760			

<sup>a</sup> An aliquot of the crystalline  $15\alpha$ -hydroxyprogesterone containing  $6.7 \times 10^4$  dpm was acetylated with  $[1-^{14}\text{C}]$ -acetic anhydride (solution 2) and the product formed was mixed with 24.3 mg of carrier  $15\alpha$ -acetoxyprogesterone prior to chromatography of the mixture on alumina and crystallization of the material eluted. The calculated specific activity was based on the dpm of  $^3\text{H}$  and weight eluted from the alumina column. <sup>b</sup> The third crystals and all of the mother liquors were combined (16.2 mg) and used for the formation of this derivative. <sup>c</sup> See footnote a of Table I.

35th week of gestation and  $5.5 \times 10^5$  dpm of purified  $[7-^3\text{H}]15\alpha$ -hydroxyprogesterone was added to the urine. The urinary conjugates were hydrolyzed with Glusulase as previously described and the neutral extract obtained weighed 3.5 g and contained  $5.2 \times 10^5$  dpm. It was chromatographed on a silica gel column and a single peak of radioactive material was eluted with 3% ethanol in methylene chloride. This material weighed 182 mg, contained  $4.26 \times 10^5$  dpm, and was further purified by chromatography on a 20-g alumina column. Elution with 1.5% ethanol in benzene gave 42 mg of material which contained  $4.0 \times 10^5$  dpm. This material was chromatographed on two sheets of Whatman paper, No. 3MM, in system C for 10 hr. The radioactive material eluted with the

mobility of  $15\alpha$ -hydroxyprogesterone weighed 11 mg, contained  $3.9 \times 10^5$  dpm, and was chromatographed on paper in system D for 5 days. Two major ultraviolet-absorbing bands were observed corresponding in mobility to  $15\alpha$ - and  $16\alpha$ -hydroxyprogesterone. The radioactivity on the paper corresponded in mobility to  $15\alpha$ -hydroxyprogesterone and it was eluted to give a residue which weighed 1.4 mg and contained  $3.4 \times 10^5$  dpm. Further purification of this material was achieved by chromatography on a 1-g alumina column, and elution with 1.5% ethanol in benzene gave an oil which weighed 0.58 mg and contained  $3.0 \times 10^5$  dpm. Crystallization of this material from acetone-ether-Skellysolve B afforded 0.11 mg of small plates containing  $1.38 \times 10^5$  dpm, mp  $230.5\text{--}232^\circ$ , mmp

230–232°, authentic 15 $\alpha$ -hydroxyprogesterone, mp 231–232°. The infrared spectrum (KBr) of this material was identical with that of authentic 15 $\alpha$ -hydroxyprogesterone as is shown in Figure 1. The isolated 15 $\alpha$ -hydroxyprogesterone had a specific activity of  $1.25 \times 10^3$  dpm/ $\mu$ g.

An aliquot of the crystals containing  $6.7 \times 10^4$  dpm was acetylated with [1- $^{14}$ C]acetic anhydride (solution 2) and the specific activities of the 15 $\alpha$ -acetoxyprogesterone and its derivative, 15 $\alpha$ -acetoxy-20 $\beta$ -hydroxypregn-4-en-3-one, were determined as described in expt 1. The specific activities obtained were constant and agreed well with the calculated values, as shown in Table IV. From the final  $^3\text{H}:^{14}\text{C}$  ratio and the specific activity of the acetic anhydride, the calculated specific activity of the urinary 15 $\alpha$ -hydroxyprogesterone was found to be  $1.30 \times 10^3$  dpm of  $^3\text{H}/\mu\text{g}$ . This value was in close agreement with the specific activity of the crystalline 15 $\alpha$ -hydroxyprogesterone isolated directly from the urine ( $1.25 \times 10^3$  dpm/ $\mu\text{g}$ ). From this specific activity it was possible to calculate that the amount of 15 $\alpha$ -hydroxyprogesterone excreted in the urine averaged 28  $\mu\text{g}/\text{day}$  over a 14-day period.

## Discussion

One of the major difficulties encountered in the isolation of 15 $\alpha$ -hydroxyprogesterone was the presence in late-pregnancy urine of 16 $\alpha$ -hydroxyprogesterone and the great similarity in chromatographic mobility of these two steroids. It is for these reasons that the addition of small amounts of [7- $^3\text{H}$ ]15 $\alpha$ -hydroxyprogesterone to the urine greatly facilitated the isolation of this steroid. The identity of urinary 15 $\alpha$ -hydroxyprogesterone was established by its melting point, mixture melting point, and infrared spectrum as compared to those of the authentic standard. Further proof of identity and its quantitation in urine was achieved by the isotope derivative studies described. The quantitation of 15 $\alpha$ -hydroxyprogesterone in late pregnancy urine was not optimal because the labeled steroid was not added in the form of the conjugate excreted. As a result, the calculated values of 34  $\mu\text{g}/\text{day}$  in expt I and 28  $\mu\text{g}/\text{day}$  in expt II must be considered as minimal titers. Experiments are now in progress to determine the nature of the conjugate of urinary 15 $\alpha$ -hydroxyprogesterone.

The only indication that neutral 15 $\alpha$ -hydroxylated steroids can be formed by mammalian tissues came from the work of Neher and Wettstein (1960) who presented chromatographic evidence for the presence of 15 $\alpha$ -hydroxytestosterone in extracts of bull testes. More recently Schneider (1965) found that liver slices of the American bullfrog (*Rana catesbiana*) was capable of converting deoxycorticosterone to a number of hydroxylated products, among which were 15 $\alpha$ - and 15 $\beta$ -hydroxydeoxycorticosterone. Schneider (1965) regarded 15 $\alpha$ - and 15 $\beta$ -hydroxylations as primitive reactions in an evolutionary sense because he could not demonstrate such hydroxylations in mammalian

tissues. With the demonstration that the adult human adrenal (Knuppen *et al.*, 1965b) and the human fetal liver (Schwers *et al.*, 1965) can 15 $\alpha$ -hydroxylate estrogens and with the isolation of 15 $\alpha$ -hydroxyprogesterone from late-pregnancy urine reported in this paper it is clear that such hydroxylations are not confined to the lower species.

The physiological significance of the presence of 15 $\alpha$ -hydroxyprogesterone in late pregnancy is not presently known. It is possible, although not as yet established, that 15 $\alpha$ -hydroxyprogesterone is elaborated in the human fetus mainly during the third trimester of pregnancy. Tweit and Kagawa (1964) showed that unsaturated, 15-oxygenated progesterones are capable of antagonizing the sodium-retaining and potassium-dissipating actions of deoxycorticosterone in adrenalectomized rats. In view of the large amounts of aldosterone secreted by the human maternal adrenal during late pregnancy (Watanabe *et al.*, 1963) and the transplacental passage of steroids (Migeon *et al.*, 1961), it is possible that the 15 $\alpha$ -hydroxyprogesterone and its metabolites, or analogous steroids elaborated in the fetus, serve to protect fetal tissues against mineralocorticoids.

## Acknowledgments

We wish to acknowledge the assistance of Dr. P. A. Diassi, Squibb Institute for Medical Research, with the microbiological preparation of [7- $^3\text{H}$ ]15 $\alpha$ -hydroxyprogesterone and for his generous gifts of steroids.

## References

- Bird, C. E., Wiqvist, N., Diczfalussy, E., and Solomon, S. (1966), *J. Clin. Endocrinol. Metab.* **26**, 1144.
- Burn, D., Petrow, V., and Weston, G. (1960), *Tetrahydron Letters* **9**, 14.
- Knuppen, R., and Breuer, H. (1964), *Z. Physiol. Chem.* **337**, 159.
- Knuppen, R., Haupt, M., and Breuer, H. (1965b), *J. Endocrinol.* **33**, 529.
- Knuppen, R., Haupt, O., and Breuer, H. (1965a), *Biochem. J.* **96**, 33C.
- Knuppen, R., Haupt, O., and Breuer, H. (1966), *Steroids* **8**, 403.
- Migeon, C. J., Bertrand, J., and Gemzell, C. A. (1961), *Recent Progr. Hormone Res.* **17**, 207.
- Neher, R., and Wettstein, A. (1960), *Helv. Chim. Acta* **43**, 1628.
- Norymberski, J. E., and Woods, G. F. (1955), *J. Chem. Soc.*, 3426.
- Ruse, J. L., and Solomon, S. (1966), *Biochemistry* **5**, 1065.
- Schneider, J. J. (1965), *Biochemistry* **4**, 689.
- Schwers, J., Eriksson, G., Wiqvist, N., and Diczfalussy, E. (1965), *Biochim. Biophys. Acta* **100**, 313.
- Tweit, R. C., and Kagawa, C. M. (1964), *J. Med. Chem.* **7**, 524.
- Watanabe, M., Meeker, C. I., Gray, M. J., Sims, E. A. H., and Solomon, S. (1963), *J. Clin. Invest.* **42**, 1619.