thereafter, and subsequently diminished. The appearance of the leucine label in colloid of all follicles examined between ½ hour and 35 hours could be accounted for by simultaneous loss of label from the epithelial cells. Since leucine is the most prominent amino-acid in thyroglobulin(3), the results are interpreted to represent the synthesis of this protein in the epithelium and its secretion into the colloid.

1. Leblond, C. P., Everett, N. B., Simmons, B., *Am. J. Anat.*, 1957, v101, 225.

2. Karpishka, Irene. Sites of protein synthesis as shown by radioautographic distribution of methionine labelled with C^{14} or S^{35} in mice and rats. M.Sc. thesis, Dept. of Anatomy, McGill Univ., 1958.

3. Haurowitz, F., *Chemistry and Biology of Proteins*, Acad. Press Inc., New York, 1950, 32.

4. Messier, B., Leblond, C. P., Proc. Soc. Exp. Biol. and Med., 1957, v96, 7.

5. Nadler, N. J., Leblond, C. P., Bogoroch, R., *Endocrinology*, 1954, v54, 154.

6. Tarver, H., Peptide and Protein Synthesis. Protein Turnover, in H. Neurath and K. Bailey, *The Proteins*. Acad. Press, Inc., New York, 1199-1292.

7. Leblond, C. P., Glegg, R. E., Eidinger, D., *J. Histochem. Cytochem.*, 1957, v5, 445.

8. Ujejski, L., Glegg, R. E., *Can. J. Biochem. Physiol.*, 1955, v33, 199.

9. Nadler, N. J., Leblond, C. P., *Brookhaven Symp. in Biol., The Thyroid*, 1955, v7, 40.

10. Wollman, S. H., Wodinsky, I., *Endocrinology*, 1955, v56, 9.

11. Gross, J., Leblond, C. P., *ibid.*, 1951, v48, 714.

Received July 5, 1960. P.S.E.B.M., 1960, v105.

6-Beta-Hydroxy-Cortisol: High Levels in Human Urine in Pregnancy and Toxemia.* (26002)

Andrew G. Frantz,† Fred H. Katz‡ and Joseph W. Jailer§

Departments of Medicine and Obstetrics and Gynecology, Columbia University College of Physicians and Surgeons, and Presbyterian Hospital, New York City

6-beta-hydroxy-cortisol (6β-OH-F) has been shown by Burstein to exist as a major metabolite of cortisol in guinea pig urine(1). He also detected its presence in minute amounts in normal human urine and in larger amounts in urine excreted during late pregnancy and after cortisol administration to normal males(2,3). Colle and Ulstrom have recently found significant quantities of 6β-OH-F in urine of the human newborn(4). The high degree of polarity conferred by the extra hydroxyl group renders 6β-OH-F difficult to extract from aqueous media with many conventional organic solvents and greatly hinders its chromatographic separation in systems designed for less polar corticosteroids. To overcome these difficulties, new methods of extraction and chromatography have been devised and the following experiments were performed using such technics.

Methods and materials. Fresh unhydrolyzed urine is extracted with ethyl acetate after prior addition to the urine of 20% by weight of sodium sulfate; this salt is also added in similar concentrations to the alkali and aqueous washes which follow. Recovery experiments and determination of partition coefficients validated the efficiency of extraction of 6β-OH-F by this method. Chromatography is carried out in 2 successive highly polar solvent systems, the first using chloroform:ethyl acetate:methanol:water; and the second, benzene:*tert.*-butanol:water. After elution from the second paper, quantitation is accomplished by the Porter-Silber reaction. Hydrolysis of the urine with β-glucuronidase before extraction did not increase the yield in several experiments. Identity and homogeneity of the 6β-OH-F recovered were confirmed by a number of technics in which an authentic sample was compared; these include chromatographic mobilities and color reac-

* Aided by Grant A-2208 (c) from Nat. Inst. Health, U.S.P.H.S.

† Trainee, Inst. of Arthritis and Metabolic Dis., N.I.H. Now on active duty, U. S. Navy.

‡ Fellow of National Foundation, 1959-60.

§ Deceased.

TABLE I. Urinary Steroids in Third Trimester of Pregnancy.

	Total P-S chromogens, μg/24 hr	No. of patients	Allo-THF, THE, μg/24 hr	No. of patients
Non-toxemic	3810 (3200-4600)*	4	2850 (1860-3230)*	4
Toxemic	4080 (1730-6500)	4	2590 (400-5160)	6
Non-pregnant	4150		3470	

* Mean and range.

tions of the isolated substance and its chemical derivatives, sulfuric acid absorption spectra, and infrared spectroscopy in some cases.

Determinations of urinary 6β-OH-F were made in 3 groups of subjects: normal men and women between the ages of 23 and 45, normal women in the third trimester of pregnancy, and pregnant women in the third trimester with pre-clampsia or toxemia of pregnancy. In addition to the 6β-OH-F determination, total corticoids were measured by the Porter-Silber reaction on another aliquot of urine following β-glucuronidase hydrolysis and ethyl acetate extraction. These latter extracts in some cases were chromatographed on a Bush B_5 system, and combined tetrahydrocortisol (THF)§, allotetrahydrocortisol (allo-THF),‖ and tetrahydrocortisone (THE)¶, were measured by methods previously described(5).

Results. 6β-OH-F values are shown graphically in Fig. 1. Mean values and ranges in μg/24 hours were: normal males, 436 (309-510); normal females, 373 (181-760); normal pregnancy, 845 (563-1320); toxemic pregnancy, 1970 (1490-2390).

Values for total Porter-Silber corticoid excretion and combined metabolites after chromatography are listed in Table I together with mean values for non-pregnant subjects obtained in this laboratory.

Other steroidal substances having similar polarities but occurring in lesser concentrations were also noted on the chromatograms. One of these was tentatively identified as an isomer of 6β-OH-F, 6α-hydroxycortisol.

Discussion. Several interesting findings emerged from these experiments. First, levels of 6β-OH-F in normal adults were considerably greater than those of any other unconjugated hormone we observed, including cortisol itself, which, as reported by others, averages about 40 μg per day(6,7).

Second, a definite rise in 6β-OH-F excretion occurs in the third trimester of normal pregnancy which is not related to any general rise in corticoid excretion, resulting in an increased ratio of 6β-OH-F to the other metabolites measured. This suggests that there is an increased conversion of endogenous cortisol to 6β-OH-F in normal pregnancy rather than an increased total production of cortisol.

Third, the increased concentrations of 6β-OH-F seen in normal pregnancy become further intensified in toxemia. A slight rise in overall Porter-Silber steroid excretion in the toxemic group, too small here to be significant, might be accounted for, if further confirmed, by increased ACTH production under conditions of stress. Both this factor and altered steroid degradation may be operating to increase the 6β-OH-F excretion in toxemia.

An alternative possibility is that 6β-OH-F arises not as a degradation product of cortisol but as an independent secretion of the adrenal gland, since incubation studies by Touchstone (8) indicate that the human adrenal gland is capable of producing this hormone *in vitro*.

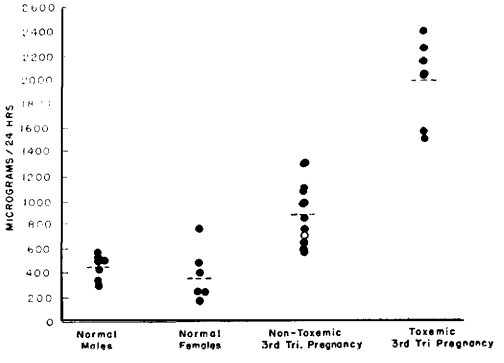

FIG. 1. Urinary excretion of 6β-OH-cortisol.

§ THF, 3α,11β,17α,21-tetrahydroxy-pregnan-20-one.

‖ allo-THF, 3α,11β,17α,21-tetrahydroxy-allopregnan-20-one.

¶ THE, 3α,17α,21-trihydroxy-pregnane-11,20-dione.

Some preliminary experiments have been carried out in feeding large doses of exogenous cortisol to subjects in each of the 3 groups studied and measuring % of dose recovered as 6β-OH-F. There was 13.5 and 16% conversion to 6β-OH-F in 2 toxemics and 13.5 and 22% in 2 non-toxemic pregnant women, while 2 non-pregnant women excreted 3.8 and 6% as 6β-OH-F. These observations indicate that there is an increased conversion of cortisol to the 6-beta derivative in both toxemia and normal pregnancy.

The biological effects of 6β-OH-F have not been fully studied, but at present there is no evidence which would assign to it a causative role in toxemia. The likelihood is that 6β-hydroxylation represents an available pathway for cortisol degradation, rendering the steroid more water soluble and suitable for excretion without the necessity for A-ring reduction and glucuronide conjugation. If the enzymatic steps concerned with either of these 2 reactions are interfered with, as might occur in some conditions, then 6-beta-hydroxylation could function as an important alternate pathway of cortisol metabolism.

Summary. 6β-OH-cortisol has been found to be the most abundant unconjugated corticoid in human urine. Normal pregnancy is accompanied by elevated levels which are further increased in toxemia. The evidence to date suggests that in these conditions an altered metabolism of cortisol takes place in which 6β-hydroxylation becomes of greater quantitative significance.

We acknowledge with thanks Dr. Seymour Bernstein's generous gift of the 6β-OH-F standard. Infrared spectroscopy was performed through the kindness of Dr. Samuel Solomon and Dr. Seymour Lieberman.

1. Burstein, S., Dorfman, R. I., *J. Biol. Chem.*, 1955, v213, 581.

2. Burstein, S., Dorfman, R. I., Nadel, E. M., *Arch. Biochem. and Biophys.*, 1954, v53, 307.

3. Nadel, E. M., Burstein, S., Dorfman, R. I., *ibid.*, 1956, v61, 144.

4. Colle, E., Ulstrom, R. A., *Am. J. Dis. Chil.*, 1959, v98, 574.

5. Frantz, A. G., Holub, D. A., Jailer, J. W., *J. Clin. Invest.*, 1960, v39, 904.

6. Jones, K. M., Lloyd-Jones, R., Riondel, A., Tait, J. F., Tait, S. A. S., Bulbrook, R. D., Greenwood, F. C., *Acta Endocrinol.*, 1959, v30, 321.

7. Peterson, R. E., Nokes, G., Chen, P. S., Jr., Black, R. L., *J. Clin. Endocrinol. and Metab.*, 1960, v20, 495.

8. Touchstone, J. C., Kasparow, M., Rosenthal, O., *Fed. Proc.*, 1959, v18, 340.

Received July 6, 1960. P.S.E.B.M., 1960, v105.

Immunological Differentiation of Human Testicular (Spermatocele) and Seminal Spermatozoa.* (26003)

ALFRED J. WEIL AND JEAN M. RODENBURG
Department of Bacteriology, The Bronx Hospital, New York 56

The seminal plasma and spermatozoa of man have powerfully antigenic material in common(1). These antigens are highly organ and species specific. The same is true for rabbit(2), guinea pig(3), bull(4), and buffalo(5). The fact that similar antigens are found in aqueous extracts of prostate and seminal vesicles, but not in those of testicle or epididymis, and that the ejaculate of azoospermic man contains these antigens, suggested that they are the product of the adnexal glands of the genital tract and that spermatozoa take up the antigens during their passage through these organs(1,2). Recently, direct evidence was obtained that spermatozoa from the rabbit's epididymis lack these antigens(6).

For men, similar evidence is difficult to obtain, because spermatozoa from testes or epididymis cannot be easily obtained in adequate numbers. An alternative source for spermatozoa, however, is occasionally available in

* Supported by grant of Nat. Inst. Health, U.S.P.H.S.