Missouri Section.

Washington University Medical School, October 11, 1933.

7052

Preparation of Gonadotropic Substance by Tungstic Acid Precipitation.

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We presented data on the quantitative precipitation of small quantities of prolan with tungstic acid.[1] Extending our work to pregnancy urine we found that tungstic acid would produce a precipitate which contained most of the gonadotropic substance and a considerable quantity of both protein and tungstic acid. Since our interest lay in the purification of the gonadotropic material, several processes for the removal of the impurities were studied. The barium precipitation used in our earlier work and which was also used for the decomposition of the phosphotungstate precipitate (Wiesner and Marshall,[2] Zondek, Scheibler and Krabbe[3] proved to be less satisfactory than the treatment with certain inexpensive alkaloids.

To one liter of urine add 20 cc. of sodium tungstate (10%). Acidify this mixture to congo red paper with sulfuric acid. Centrifuge and wash the precipitate once with acetone. A little distilled water and some solid brucine are added, the mixture thoroughly stirred and then centrifuged. The clear supernatant solution contains nearly all of the gonadotropic substance of the original urine.

Although our experience is somewhat limited it seems probable that sodium molybdate may be used instead of sodium tungstate. Quinine and possibly benzidine hydrochloride or 8, hydroxy-quinoline may be used in place of the brucine. Barium hydroxide gives less satisfactory preparations and sodium hydroxide or aqueous pyridine dissolves nearly all of the tungstate precipitate.

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[1] Katzman, P. A., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1188.

[2] Wiesner, B. P., and Marshall, P. G., *Quart. J. Exp. Physiol.*, 1931, **21**, 147.

[3] Zondek, B., Scheibler, H., and Krabbe, W., *Biochem. Z.*, 1933, **258**, 102.

Since we have found that some purified preparations of the gonadotropic substance fail to give a precipitate with tungstic acid, it is probable that the active material is carried down by adsorption. The function of the brucine is 2-fold; to release the gonadotropic material from the precipitate and to form an insoluble tungstate which prevents the tungsten from dissolving. As shown

TABLE I.
Gonadotropic Substance Obtained from Urine by Different Procedures. Rat Units per Liter of Urine.

Urine No.	Benzoic Acid Method	Tungstic Acid with NaOH	Tungstic Acid with Brucine	Tungstic Acid with Quinine
118	<160	>4430<5175	8300	>4500
119	170	6000	>5940<7180	>4690
120	<460	5500	>6600<7800	>3300<4980
121	>1000<1200	>2750<4150	2970	<2500
122	>1050<1360	>5840<7700	>6380<7000	>6800
123	>1200<1500	>4600<5940	5810	5810
124	1750	7100	6640	
125	>700<1000	6600	6500	
126	>570<1140	3819	>2750<4150	
127	<900	>3300<4400	>4150<5500	
128	850	5540	>4950*	

*Using hydroxy-quinoline instead of brucine >2575<4300 R. U.

in the table, the tungstic acid procedure gives much higher yields than those obtained by the benzoic acid method (Katzman and Doisy[4]). The yields obtained by the use of brucine or quinine compare favorably with those of the crude sodium hydroxide solutions of the tungstate precipitate.

The small amount of brucine present in these extracts may be removed by extraction with chloroform after the addition of dilute sodium hydroxide to ensure that the alkaloid is present in the free form.

The data on the tungstic acid procedure given in the table were obtained with 500-1000 cc. samples of urine, while the yields by the benzoic acid method are based on results from 100-200 liter batches. Preliminary experiments indicate that the new process will work satisfactorily on large volumes of urine.

[4] Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1932, **98**, 739.