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Method for Concentrating the Gonadotropic Activity in Pregnancy Urine.*

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For the past several years we have employed a procedure for concentrating the gonadotropic substance in the urine of pregnant women which has yielded an excellent extract for general physiological purposes. The method is not difficult to carry out, is quantitative and the product is non-toxic even though administered in massive doses. The procedure is based on the fact that the gonadotropic material from pregnancy urine is precipitated by tannic acid, and by treating the tannic precipitate with pyridine the active principle is again set free from the tannic acid and becomes water soluble.

An aqueous concentrate of tannic acid is added to the urine until no further precipitation takes place and, after standing in the cold for several hours, the precipitate is collected by suction filtration, washed with acetone and powdered. The powder may be stored as such and has been kept for as long as 3 years without detectable loss in activity. For further purification, the powder is extracted 8 to 10 hours with constant stirring with 50% aqueous pyridine (200 cc. per 10 gm.—the equivalent of about 5 liters of the original urine). The residue is removed by centrifuging and re-extracted with a like amount of pyridine solution for the same length of time. The two solutions are united and evaporated in a warm current of air (40°C.) or precipitated by pouring into 10 volumes of acetone, adding a small amount of saturated sodium chloride or sodium acetate solution. The residue is then extracted 4 hours with from 10 to 15 cc. distilled water 5 different times, centrifuging each time (cooling the solution to freezing just before centrifuging aids in throwing down the precipitate). All of the solutions are united and the activity precipitated by adding 10 volumes of acetone. The precipitate, which contains the activity, is removed by centrifuging and again extracted 5 times with from 3 to 5 cc. of water each time and again precipitated with 10 volumes of acetone. The resultant precipitate is readily soluble in a volume of water representing a 500-fold concentration of the original urine. As much as 6 cc. of

* Aided by a grant from the National Research Council, Committee on Problems of Sex, administered by F. L. Hisaw.

the extract (3 l. urine equivalents), administered within 3 days into immature rats and 2 cc. per day injected intravenously into sexually immature monkeys for 10 days, is not toxic.

TABLE I.

The Ovarian Weights of Immature Rats, 22 Days Old, after Injection of Definite Amounts of 3 Different Urine Samples, Variously Treated. The Vaginæ of All Test Rats Were Open at the Time of Autopsy.

Urine sample	Amt.	Ov. wts. in mg. after treatment with: Whole urine	Tan. Ppt.	Extr.	Diluted 1:4 Nor. Ur.	Dil. 1:4 Boiled Ur.	Dil. 1:250 Boiled Ur.
1	2 cc.	27	28	26.4			
		26	29	25.5			
			25				
2	1 cc.	22	21	20			
		20	21.5	23			
3	2 cc.	23	27.5		23	27	
		34	28		28.5	24.5	
		21.5	25.5		29	26.5	
		23.5					
		22.5					
	.5 cc.*	16	20.5	17.5	18	19	17
		18	16	16	19.5	16.5	15.5
		16.5	16.5	17	17.5	18.5	18
							16

* Ovarian weights of uninjected controls 11 to 14 mg.

Table I presents the resultant weights of immature rat ovaries after administration of 3 different urine samples. The urines were first standardized by direct injection after extracting with ether to remove the oestrin. They were then precipitated with the tannic acid and both the crude precipitates and purified extracts were assayed. The method is quantitative since the purified extract in comparable doses is as active as the original urine. To further test the completeness of the precipitation and of the extraction, the supernatant urine after tannic acid treatment was precipitated with 10 volumes of acetone and this precipitate in 250 cc. equivalent amounts induced no stimulation in immature female rats; likewise the pyridine and water insoluble fractions in the same equivalent amounts were without effect.

To find whether the method would give quantitative results for less concentrated solutions, aliquot portions of pregnancy urine (Sample 3) were diluted 4 times with normal and boiled male urine, precipitated, and extracted in the usual manner. Another portion of the pregnancy urine was diluted 250 times with boiled male urine and extracted. It can be seen in the table that total recovery of gonadotropic activity was accomplished in each instance.