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**Improved Procedure for Biological Titration of Estrogenic and Gonadotropic Hormones in Sera of Pregnant Women.**

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Although some studies have been reported on the concentration of estrogenic and gonadotropic substances in whole blood and serum during pregnancy, their number and accuracy have been limited by the large quantities of blood necessary for the biological tests employed.

By using unextracted sera as the test solutions, rather than extracts of blood or sera which contain only a fraction of the hormones, and mice, rather than rats, as the test animals, it was found that reliable results could be obtained with from 20 to 50 cc of blood.

*Estrogenic Hormone.* A modification of Fluhmann's technic<sup>1</sup> was employed. Whole or diluted serum was injected subcutaneously in 6 divided doses over a period of 3 days into castrated mice of approximately 8 weeks of age and 25 g in weight, beginning on the 8th day after oöphorectomy. The animals were sacrificed on the 4th afternoon. Biopsies were taken from the middle portion of the vagina and histologic sections prepared by a rapid technic. The least amount of serum which produced complete hyperplasia to vaginal cornification (reactions 4 to 5, Fluhmann) were regarded as containing 1 mouse unit. Also the uterine cornua were examined macroscopically for estrus, since macroscopically positive uteri indicated dosages several times the amount necessary to produce vaginal cornification.

For the initial titration 3 to 6 mice were injected with varying amounts of sera. When the approximate quantity necessary to produce cornification was found, 3 to 6 animals were injected with more closely grouped dosages. Further titrations were conducted if necessary. Six to 12 animals were generally used for each titration, while 3 to 6 animals were injected with amounts close to the finally accepted value.

The values obtained in 88 determinations on 26 pregnant women (Table I) were approximately 20 times greater than those of Smith,<sup>2</sup> who used ether extracts of whole blood.

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<sup>1</sup> Fluhmann, C. F., *Endocrinology*, 1934, **18**, 705.

<sup>2</sup> Smith, M. G., *Bull. Johns Hopkins Hosp.*, 1927, **41**, 62.

TABLE I.  
Serum Estrogen and Prolan in Normal Pregnancy.  
(Eighty-eight determinations on 26 patients.)

Wks of Gestation	Sera Titrated	Estrogenic Hormone			Gonadotropic Hormone		
		Avg	—Range—		Avg	Low	High
			Low	High			
6-7	4	—	—	25	575	330	800
8-9	6	24	16	33	760	400	1660
10-11	8	36	20	60	850	400	2000
12-15	10	43	16	100	960	300	2000
16-19	10	87	33	160	510	200	1000
20-23	9	131	100	200	211	120	300
24-27	11	168	80	330	160	100	250
28-31	11	185	120	380	135	250	100
32-35	9	313	120	500	172	100	300
36-39	10	430	300	800	185	100	330

*Gonadotropic hormone.* A modification of the Ascheim-Zondek test was employed. Whole or diluted sera were injected in 6 divided doses over a period of 3 days into infantile mice approximately 17 days old and 8 g in weight, and the animals were killed on the 5th morning. The least amount which caused the production of corpora hemorrhagica or corpora lutea in 2 of 3 animals was considered as containing one mouse unit. The ovaries were inspected with the aid of a hand lens, but any suspicious specimens were sectioned for histologic examination. It was found expedient to recognize either corpora lutea or corpora hemorrhagica as end points, because in some specimens the amount of follicle-stimulating hormone was equal to or greater than the luteinizing fraction, although it must be recognized that their significance may prove to be quite different.

The end point was most conveniently approximated by making a series of preliminary titrations using single animals for a number of widely separated dosages. Final titrations were conducted on groups of 3 animals.

A composite graph made from the titration values of 88 specimens (Table I) reveals a curve similar to that obtained from extracts of the serum by Boycott and Rowland<sup>3</sup> and Smith and Smith.<sup>4</sup> The latter workers state that they employed extracts of the sera and urines for titrations of gonadotropic substances because the unextracted material was toxic for immature animals. This difficulty was rarely experienced with the small quantities of sera required for each test during pregnancy. Whole sera yielded values many times higher than extracted specimens.

<sup>3</sup> Boycott, M., and Rowlands, I. W., *Brit. M. J.*, 1938, 1, 1097.

<sup>4</sup> Smith, G. V., and Smith, O. W., *Am. J. Phys.*, 1934, 107, 128.