

Augmentation of the Pregnant Mare Serum Gonadotropic Effect.

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It is now generally recognized that the physiological effects observed on administration of the purified gonadotropin from pregnant mare serum into hypophysectomized immature female rats at different dose levels are: Minimal doses produce only repair of the deficient interstitial tissue without any apparent effect either upon the follicles or indirectly upon the vagina and uterus. Larger amounts, in addition to the repair of the interstitial tissue, also cause development of follicles. A further increase in dosage results in the formation of corpora lutea and estrous changes in the vagina and uterus.^{1, 2}

It is at present not possible to decide whether the gonadotropic effect of pregnant mare serum is exerted by two distinct separate principles, one specifically affecting the interstitial tissue of the ovary and the Leydig tissue of the testis, and the second causing follicular growth in the female and germinal tissue development in the male, or whether it is the property of a single individual principle. No pure crystalline gonadotropic pregnant mare serum compound possessing both, or any one of the activities has as yet been isolated. The possibility has to be considered that the two principles (follicle-stimulating and interstitial cell-stimulating) may be linked together in the form of a single composite molecule ("mother molecule") or that they may be attached to the same carrier protein. This theory does not necessarily mean that the two "factors", if combined in a single molecule or if attached to the same carrier protein, are present in the most favorable ratio for complete synergism in the rat, or that the optimum proportions are the same for all species of animals, or for both sexes of any one species.

It seemed of interest to determine whether the gonadotropic response of pregnant mare serum, as tested by the increase of ovarian weight in normal immature female rats, could be augmented either by the addition of a follicle-stimulating or interstitial cell (luteinizing) preparation.

Experimental. The gonadotropic preparations used in this study

¹ Leatham, J. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **42**, 590.

² Cole, H. H., Pencharz, R. I., and Goss, H., *Endocrinol.*, 1940, **27**, 548.

were a pregnant mare serum fraction (P.M.S.) assaying 1800 international units per mg, a follicle-stimulating preparation (F.S.H.) obtained from sheep pituitary, containing 40 rat units* per mg, and a chorionic gonadotropin preparation from human pregnancy urine containing 200 international units per mg.

The augmentation experiments were carried out in normal immature female rats (21-23 days old) by subcutaneous administration of the gonadotropic pregnant mare serum preparation alone or of *in vitro* combination of this preparation with either the follicle-stimulating preparation or with chorionic gonadotropin. Injections were made once daily for 3 days, followed by autopsy 24 or 48 hours after the last injection. The animals used in these experiments were either of the Sprague-Dawley or Long-Evans strains. The data given in Table I represent average results obtained with about 20 animals per group (Series 1) and 10 animals per group (Series 2).

From the results recorded in Table I, it is apparent that the addition of a follicle-stimulating anterior pituitary fraction enhances the gonadotropic activity of pregnant mare serum as measured by the increase in ovarian weight. The follicle-stimulating hormone preparation employed in such augmentation experiments naturally has to be comparatively free of the interstitial cell-stimulating or luteinizing principle. As can be seen from Table I, no true augmentative response was observed when pregnant mare serum was administered in combination with chorionic gonadotropin.

TABLE I.
Effect of *in Vitro* Addition of Either Follicle-Stimulating Hormone or Chorionic Gonadotropin to Pregnant Mare Serum Gonadotropin.

Series No.1				Series No.2		
Dosage				Dosage		
P.M.S., I.U.	F.S.H., R.U.	Chorionic Gonadotropin, I.U.	Ovarian wt, mg	P.M.S., I.U.	F.S.H., R.U.	Ovarian wt, mg
8	—	—	23	5	—	22
16	—	—	47	10	—	31
—	4.0	—	22	—	4.8	27
—	—	20	29	—	3.6	20
8	4.0	—	80	5	4.8	88
16	4.0	—	100	10	3.6	117
8	—	20	35	—	—	—
16	—	20	62	—	—	—

Series No. 1 autopsied 24 hours after the last injection. Series No. 2 autopsied 48 hours after the last injection. Uninjected controls ovarian weight 13 mg.

* One rat unit of F.S.H. capable of causing resumption of follicular growth in immature hypophysectomized rats.

The augmentation of pregnant mare serum gonadotropin by the pituitary follicle-stimulating hormone can be considered to be of a specific nature, since it has been shown that non-specific augmenting substances, such as copper or zinc salts, inert proteins, adrenalin, etc., which synergize pituitary extracts, do not augment the hormone from pregnant mare serum.³

Summary. An augmentative effect was observed when pregnant mare serum gonadotropin was administered in combination with a pituitary follicle-stimulating preparation. On the other hand, the addition of chorionic gonadotropin under the same experimental conditions, did not potentiate the gonadotropic effect of the pregnant mare serum preparations.

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Electrophoretic Analysis of Anti-Papilloma Rabbit Serum.*

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Serum from domestic rabbits bearing the growths of infectious papillomatosis¹ and those injected with extracts of domestic or cottontail rabbit growths or with the purified papilloma virus protein may contain immune bodies demonstrable by complement fixation, neutralization or precipitation.² Recently, studies have been made in this laboratory to learn whether these immunological characters are paralleled by abnormal electrophoretic behavior of such serum.

Sera from individuals of 6 groups of rabbits were examined: (1) domestic rabbits injected intraperitoneally with 0.5 mg of purified papilloma virus protein, once a week for 6 weeks, and serum taken for study every 7 days (for a total of 51 days); (2) domestic rabbits injected intravenously with 0.5 mg of purified papilloma virus protein, twice a week for 6 weeks, and the serum examined after 21 and 50 days; (3) domestic rabbits injected intraperitoneally with

³ Evans, J. S., Hines, L. R., Ceithaml, J. J., and Koch, F. C., *Endocrinol.*, 1940, **26**, 1012.

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¹ Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607.

² Bryan, W. R., and Beard, J. W., *J. Nat. Cancer Inst.*, 1941, **1**, 607.