gonadotropic injections were influenced in the same direction, and to about the same degree as those influenced by beef testes. They fell short of being fully of the hen type. Following removal of the grafted testis new feathers were of the capon type, similar to those developed before testicular grafting or injection. Following a second testicular graft, feathers completely of the hen type were produced by both grafted and host skin.

These observations are essentially in accord with the findings (but not necessarily with the conclusions) of most students of this subject. It appears to the present writer that there are probably both somatic and endocrine variables, that the testicular secretion of hen-feathered and cock-feathered male are not precisely equivalent, at least quantitatively, and that thresholds of response are not so distinct as has sometimes been supposed. Further, the rather similar manifestations following injection of mammalian testicular extract and excessive stimulation of an avian testicular graft does not afford evidence of sufficient delicacy to warrant the conclusion that the bull and cock produce identical testicular hormones.

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Two Gonadotropic Substances in Mare Serum.*

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The finding by Cole and Hart[1] that there is no relationship between the activity of mare serum when administered alone and its activity when combined with pituitary synergist,† led them to believe that there are two gonadotropic substances in mare serum. More convincing evidence is now at hand. We have been able to make potent pregnancy serum completely ineffective when given alone without destroying its synergistic properties. This has been

* The chemical work described here was carried out in the Department of Biochemistry of the University of California under the supervision of Dr. D. M. Greenberg.

1 Cole, H. H., and Hart, G. H., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 370.

† This pituitary synergist was prepared after the method of Evans *et al.*[2] and probably is an impure preparation of the follicle-stimulating hormone of Fevold and Hisaw.

2 Evans, H. M., Simpson, M. E., and Austin, P. R., *J. Exp. Med.*, 1933, **58**, 545.

accomplished by several diverse methods. After treatment with H_2S and subsequent incubation at 37°C., the serum when given alone has no effect on the ovaries of immature rats, but when this serum is combined with pituitary synergist, marked augmentation occurs as is shown in Table I.

TABLE I.

Material		Amount injected	Ovarian weight, mg.
Serum treated with H_2S not incubated		1 cc.	163
" " " H_2S and "		1½ cc.	13
Pituitary synergist		2 mg.	25
Serum treated with H_2S and incubated	combined	1½ cc.	
Pituitary synergist	combined	2 mg.	48

We interpret this as meaning that one substance has been destroyed by this treatment. The substance destroyed was capable of affecting the ovary either alone or in conjunction with the luteinizer remaining in the serum after treatment.

We have attempted to separate the 2 hormones from mare serum without destruction of either as has been done so successfully with the pituitary by Fevold and Hisaw.[3] They have shown that the pituitary contains 2 gonadotropic substances, a follicle-stimulator and a luteinizer. According to them the follicle-stimulating hormone will give a moderate response consisting of follicular development when given alone and the luteinizer gives no increase in ovarian weight, whereas if the 2 are combined the response is augmented. By Fevold's method, as well as by the method just described, we have succeeded in obtaining fractions of mare serum which behave similarly to pituitary luteinizer; *i. e.*, the fraction when administered alone gives no response but if united with pituitary synergist, the response of the latter is augmented. This is shown in Table II.

TABLE II.

Material		Amount injected mg.	Ovarian weight mg.
Mare serum luteinizer		10	18
Pituitary synergist		2	25
Mare serum luteinizer	combined	5	63
Pituitary synergist	combined	2	

We were also able to effect a crude concentration of the luteinizing substance by salting out, a method suggested by the work of

[3] Fevold, H. L., and Hisaw, F. L., *Am. J. Physiol.*, 1934, **109**, 655.

Goss and Cole.[4] We separated out 4 fractions of serum. All of these stimulated the ovaries of immature rats when given by themselves but only one of these fractions, that salted out by the addition of between 14% and 18% Na_2SO_4, was capable of augmenting the effect of pituitary synergist, showing that most of the luteinizing substance was contained in this fraction.

Thus, our data show that pregnant mare serum contains a luteinizer‡ identical, or at least very similar, to that occurring in the pituitary together with a second gonadotropic hormone which is destroyed after treatment with H_2S and subsequent incubation. We have not been able to identify this second substance as the follicle-stimulating hormone of Fevold and Hisaw. None of our fractions have given a pure follicle-stimulating response and we have obtained only slight evidence of augmentation when serum luteinizer has been combined with other mare serum fractions. In fact, we have not been able to differentiate the response of serum fractions which are effective when administered alone from the response of untreated serum. Our failure to obtain a fraction of mare serum having biological properties comparable to those of the follicle-stimulating hormone of Fevold and Hisaw may be explained by assuming either that the second gonadotropic substance in mare serum is not identical to the follicle-stimulating hormone obtained from the pituitary or that a follicle-stimulating substance similar to that found in the pituitary is present but not recognized because of contamination with serum luteinizer. Our data would indicate that the first assumption is the correct one.

[4] Goss, H., and Cole, H. H., *Endocrinology*, 1931, **15**, 214.

‡ This so-called serum luteinizer is probably also identical to the substance which Evans *et al.* found in the sera of non-pregnant animals and referred to as a "prolan-like" substance.