

TABLE I.
Inactivation of Thyrotropic Hormone from Different Species of Animals Produced in Guinea Pigs by the Serum of a Dog which Had Been Injected for Sixteen Months with a Sheep-Pituitary Extract (No. 1).

Source of Hormone	Thyroid glands of guinea pigs: Hormone alone		Thyroid glands of guinea pigs: Hormone plus Serum	
	Wt.	Activity	Wt.	Activity
Extract No. 1 (Sheep), 1.0 cc.	56	Activated 2 plus	24	Inactivated
Extract No. 2 (Sheep), 0.5 cc.	81	" 3 "	25	"
Extract No. 3 (Beef), 0.5 cc. (Looser)	75	" 3 "	17	"
Extract No. 4 (Beef), 1.0 cc. (P. D. & Co.)	96	" 4 "	47	Partially inactivated
Extract No. 4 (Beef), 0.25 cc. (P. D. & Co.)	63	" 3 "	29	Inactivated
Extract No. 5 (Man), 3.0 cc.	25	" 1 "	25	"

Summary. Daily injections of sheep-pituitary extract into a dog produced an antihormone which inactivated in guinea pigs substantial test doses of thyrotropic hormone from sheep and bovine pituitary glands and from human urine of myxoedema. Injections of the serum alone into guinea pigs produced upon their thyroids an effect which resembled hypophysectomy.

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The Augmentary Factor in Animal Sera After Injections of Pituitary Extract.*

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There has recently appeared a most timely report of the preparation of a pituitary gonadotropic antagonist by Evans, Korpi, Pencharz, and Simpson.¹ According to these workers this pituitary fraction, representing what Sharpey-Schafer would have called a chalone, is probably present in considerable amounts in most crude pituitary extracts, and accounts for the strange lack of activity shown by certain gonadotropic extracts from which greater potency would have been expected. Such an extract has been the subject of inves-

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¹ Evans, H. M., Korpi, K., Pencharz, R. I., and Simpson, M. E., *Univ. of Calif. Pub. in Anat.*, 1936, 1, 237.

tigation in this laboratory in experiments where the development of gonadotropic antihormones in animal sera was studied during many weeks of its injection.

The purpose of the present communication is to record the facts which lead to the following conclusion, *viz.*, that the results of the experiments are best explained by assuming the formation in the injected animal of an antihormone to the antagonist.

The injected extract† was prepared from sheep-pituitary glands by a method slightly modified from that described by VanDyke and Wallen-Lawrence.² The animals injected were 2 large dogs, a male and a female, and a young mare. They received the following daily dosage of the extract: the male dog, 5 cc.; the female dog, 25 cc.; and the mare, 50 cc. The animals were bled before the injections were started, after which time the dogs were bled every week, and the mare approximately every 20 days.

The sera were each tested in 25-day-old rats for ability to modify the gonadotropic effects of the extract, as follows: (1) the standard activity of the gonadotropic extract *alone* was determined by giving to a group of rats 3 subcutaneous injections of the extract on each of 2 successive days, the rats being sacrificed one hundred hours after the first injection. (2) The tests for the combined effect of serum and extract were coincidentally made in other groups of rats which were given serum twice daily beginning 2 days before the injections of extract were begun and continuing through that period. Care was taken to inject the serum into one side of a rat and the extract into the other side, so as to avoid mixing the materials at the site of injections. The average weight of the ovaries of each group of rats was used as a measure of the gonadotropic effect.

The normal sera had very little effect upon the gonadotropic action of the pituitary extract in the rats. After 20-30 days of injections the serum of each of the 3 animals began to reveal an unsuspected ability to augment the gonadotropic action of the extract. The augmenting power of the sera reached a peak after 50-80 days of injections, at which time the gonadotropic action of the extract was enhanced three-fold. This remarkable power of augmenting gonadotropic action was rapidly lost by both the bitch and the mare after

† For several reasons, some of which were discussed in the report of Evans and his co-workers, the suspicion has long been entertained by us that this extract contained an antagonist in addition to a substance which gave rise to antihormones.

² VanDyke, H. B., and Wallen-Lawrence, Z., *J. Pharmacol. and Exp. Therap.*, 1933, **47**, 163.

70-90 days of injections, at which time moderate doses of either of these 2 sera completely inactivated the extract. The serum of the male dog, which received the smallest dose of extract, continued to augment the gonadotropic action of the extract after 130 days of injections, when this report was written. The results of the tests are illustrated in Fig. 1. The augmenting serum transformed the rather weak gonadotropic extract into an exceedingly potent one, whose effects on the ovary resembled those of a highly active preparation of the serum of pregnant mares.

The nature of the augmenting action of the sera. This question was immediately raised, and the possible causes of the phenomenon,

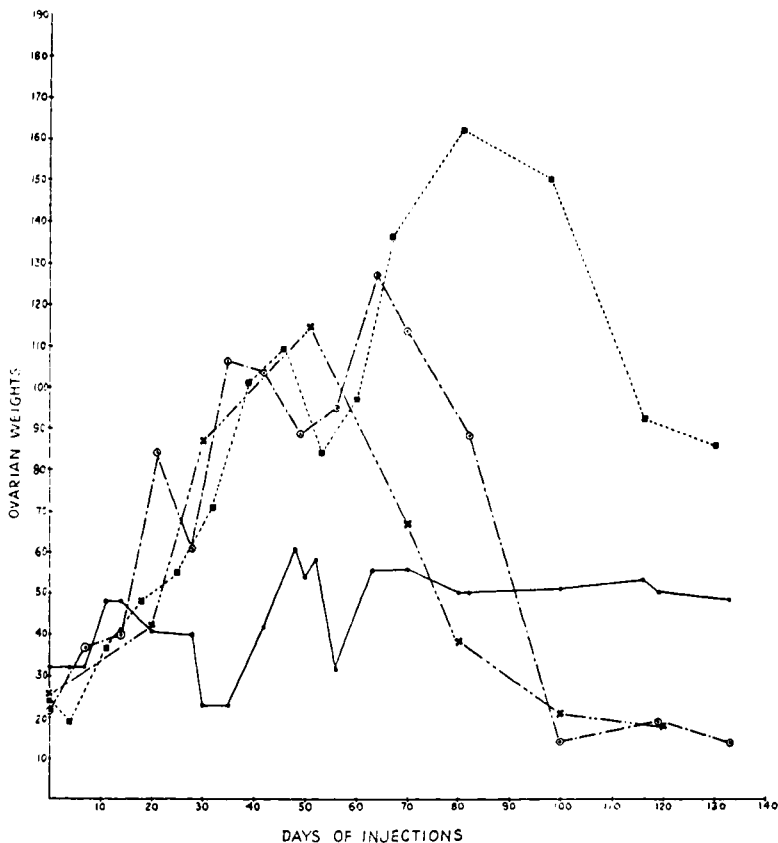


FIG. 1.

The development in the sera of the factor which augmented in test animals the action of pituitary gonadotropic extract. The average weight of the ovaries of rats tested with serum plus extract, and with extract alone, are plotted in relation to the day when serum was taken for each of the combined tests. The dotted lines represent the effect of the sera upon the potency of the extract. The unbroken line represents the potency of the extract alone at the time of each test.

so far as could be seen, were tentatively listed as follows: (1) the presence of F.S.H. in the sera; or (2) a delayed absorption of the gonadotropic hormone with a more gradual action possibly produced by the serum, permitting augmentation in the manner of the tannate or protamines of insulin which supposedly delay absorption and thereby spread the action of the hormone over a greater period of time; or (3) the presence in the sera of an antihormone to the antagonist which, by holding in abeyance both the antagonist in the tested rat and in the injected pituitary extract, gave complete freedom of action to all the gonadotropic hormone.

Further experiments designed to shed light on the subject were made as follows: First, the sera were tested alone as for F.S.H., in hypophysectomized immature rats. These animals were hypophysectomized at 25 days of age, and 5 days later the tests were begun. In no instance were there signs of stimulation of the ovaries; in fact, the tested hypophysectomized rat ovaries tended to be slightly smaller and less active in appearance than those of the normal uninjected controls.

Second, the same tests of the sera alone as for F.S.H. were repeated in normal immature rats. When read at 72 hours, an effect similar to that of F.S.H. was noted; in each animal so tested definite follicles developed in the ovaries, which were nearly twice normal size.

The failure of the augmenting principle contained in the sera to stimulate the ovaries of hypophysectomized immature rats served to differentiate this principle from F.S.H.

In normal immature rats the injected serum apparently released F.S.H. present in the pituitary glands of these animals, since the serum in itself having no pituitary-like action produced an effect identical with that of F.S.H.

What indicated that a delay of absorption of the gonadotropic extract was not responsible for the enhancing effect was the fact that with the technique of the tests remaining the same, the sera produced an increasing augmentation from week to week, and ultimately caused inhibition of the extract rather than augmentation.

An antihormone to the antagonist cannot be excluded at the present time as the cause of the augmentation of the gonadotropic extract by the sera. The following facts appear to support this assumption: (1) the extract injected has readily produced antihormone to the gonadotropic and thyrotropic hormones in several species of animals; (2) the augmenting factor was found only in the pseudo-globulin fraction of the sera, the same fraction that

contains the immune bodies‡ (precipitation between 33% and 50% saturation with ammonium sulfate); (3) the factor in the sera which inactivated gonadotropic hormone and prevented further augmentation, is usually described as an antihormone, and was also found only in the pseudo-globulin fraction; and (4) the sera, which did not act upon the ovaries of the hypophysectomized rats, apparently released follicle-stimulating hormone already present in the animals, so that the normal rat ovaries were stimulated. The same effect would have been produced if the antagonist of Evans and co-workers had been inactivated in these animals.

Whether this supposed antihormone inactivated antagonist of both the rat and the extract injected into the rat, or only that of the rat itself, remains to be investigated. The rat ovary, released from antagonist, and stimulated by F.S.H. from its own pituitary, undoubtedly reacts as if augmented after injections of gonadotropic extracts that are rich in the luteinizing factor and poor in F.S.H. This sort of augmentation by the interaction of these gonadotropic fractions is now well known, and probably was a factor in the present experiments. It would seem, however, that the greater effect of removal of antagonist in the injected extract may also have been effected by the sera, since the augmentation was so marked. It will be recalled that Evans and his co-workers stated that their pituitary extracts were enhanced in activity three-fold by the removal of the antagonist. The sera of our studies at their greatest potency produced the same degree of augmentation.

Summary. Injections of a gonadotropic extract of sheep-pituitary glands into a horse and 2 dogs induced the formation in their sera of a factor which augmented three-fold the gonadotropic activity of the extract when tested in immature rats. The augmenting factor had no gonadotropic action in hypophysectomized immature rats, but produced in immature normal rats an effect resembling F.S.H. or synergist. The augmenting factor, possibly an antihormone to the pituitary gonadotropic antagonist, was present only in the pseudo-globulin fraction of the sera, which fraction also carries the immune bodies and other antihormones.

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‡ From unpublished experiments by Thompson and White.