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Studies on Gonadotropic Antihormones.

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This work was originally begun with the idea of determining, if possible, the nature of the "inhibiting substance" which Collip *et al.*,¹⁻⁵ have described and designated as "antihormones." The controversy which has arisen involves the question as to whether the action of the "antihormone" may not in reality be due to an antibody developed against the protein, which is always found in hormone preparations derived from human or animal source. It is our belief that the gonadotropic inhibiting substance is separate and distinct from the antiprotein and both are present in the antisera obtained from rabbits which have been persistently injected with large doses of pituitary extract or P.U.* hormone.

One male and 3 female adult rabbits were injected over a period of 3 weeks, receiving 5 cc. of pituitary extract (E. R. Squibb and Sons) daily, totaling 900 growth units each. The antisera, inactivated at 56° for ½ hour, gave a precipitin reaction with pituitary extract. On testing the pituitary extract for gonadotropic hormone, we found that 1/6 cc. injected intravenously into a female† rabbit gave corpora lutea. With the addition of 1 cc. of the antiserum to 1/3 cc. of pituitary extract, a heavy precipitate formed. The supernatant along with the precipitate was injected intravenously into an immature female rabbit. Examination of the ovaries 48 hours later showed no stimulation. Similar results were obtained with the other 3 antisera.

We attempted to remove the anti-bovine protein present in our pituitary antisera by treatment with normal bovine serum. Two cc. of bovine serum (1-100) incubated 2 hours with 2 cc. of pituitary antiserum gave a heavy precipitate which was centrifuged out.

¹ Collip, J. B., *J. Mount Sinai Hosp.*, 1934, **1**, 28.

² Collip, E. M., and Collip, J. B., *Lancet*, 1934, **1**, 784.

³ Selye, H., Bachman, C., Thompson, D. L., and Collip, J. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **31**, 1113.

⁴ Bachman, C., and Collip, J. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 561.

⁵ Bachman, C., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **32**, 851.

* P.U. = Pregnancy urine—gonadotropic fraction.

† Female rabbits 10 to 12 weeks of age and from a single source were used for testing purposes.

The supernatant when tested with bovine serum in dilutions up to 1-10,000 gave no precipitin reaction, indicating that the anti-bovine protein had been eliminated. Two cc. of antiserum so treated was incubated for 1 hour with 1/3 cc. of pituitary extract. The mixture was injected intravenously into a female rabbit and no ovarian stimulation was noted 48 hours later. This same result was obtained with each of the 3 antisera so treated. Precipitation of anti-bovine protein thus does not remove the inhibitory factor (antihormone) from solution.

A mixture containing 1 cc. of pituitary antiserum and 15 rat units of antophysin‡ (Winthrop Chemical Co.) was incubated 1 hour and then injected intravenously into a female rabbit. Forty-eight hours later, autopsy showed numerous corpora hemorrhagica in the ovaries, indicating that pituitary antiserum did not inhibit the action of P.U. under the conditions of the experiment.

In testing both antophysin and follutein§ (E. R. Squibb and Sons) we found that 15 R.U. injected intravenously into immature rabbits gave a good Friedman test 48 hours later. Two female rabbits were each injected with 13,000 R.U. of antophysin and 2 other female rabbits were injected with 12,000 R.U. of follutein over a period of 3 weeks. Each antiserum gave a precipitin reaction with normal male human serum and with its own antigen. One cc. of antophysin antiserum was incubated one hour with 25 R.U. of antophysin and injected into an immature female rabbit. Autopsy 48 hours later revealed normal unstimulated ovaries. A similar result was obtained when follutein antiserum plus 25 R.U. of follutein was tested.

Two cc. of antophysin antiserum was incubated 2 hours with 2 cc. of normal male human serum (1-100). A heavy precipitate formed which was centrifuged out. Subsequent precipitin reactions between the supernatant and normal human serum in dilutions up to 1-10,000 were negative, indicating that the anti-human protein factor had been removed. Two cc. of this treated antiserum was incubated 1 hour with 25 R.U. of antophysin and injected intravenously into a female rabbit. No ovarian stimulation was noted 48 hours later.

The above experiment indicates that after absorption of the antiserum with human serum protein, a factor remained in the supernatant fluid which was still capable of inhibiting the action of the hormone used to produce this antiserum. This was not due to the

‡ Antophysin = P.U. extract.

§ Follutein = P.U. extract.

antiprotein factors since, as far as we could determine these had been removed by absorption.

In testing the action of antophysin antiserum on pituitary extract, 1 cc. of the antiserum was incubated 1 hour with 1/3 cc. of the extract and the mixture injected into a female rabbit. There was no ovarian stimulation 48 hours later. A similar result was obtained when follutein antiserum was tested against pituitary extract. The pregnancy urine *antiserum* inhibited the gonadotropic action of pituitary extract as well as P.U.; whereas pituitary antiserum (as shown above) inhibited the gonadotropic action of only the pituitary extract. We feel that there was not sufficient gonadotropic "antihormone" developed to inhibit the hormone action of P.U.|| Our assay of pituitary extract for gonadotropic hormone revealed 20 R.U./cc.; thus the rabbits injected with pituitary extract received only a total of approximately 1750 R.U. of gonadotropic hormone.

The fact that our P.U. antisera, after treatment with human serum proteins, will still inhibit the hormone action of P.U. is a significant finding; and since these antisera will also inhibit the hormone action of pituitary extract derived from bovine source, it thus appears that the antihormone (inhibitory action) is not due to an anti-human protein phenomenon *per se*, but to the presence of another antagonistic but specific substance.

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Cataleptic Symptoms Following Bilateral One-Stage Removal of Frontal Cortex in Cats.

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The author wishes to present a brief report concerning a peculiar group of symptoms, in many ways suggestive of catalepsy, which has been observed in 8 cats following a one-stage operation in which both frontal lobes of the cerebral cortex were removed. These operations were done with every precaution to secure good hemostasis and prevent hemorrhage from complicating the interpretation of the subsequent observations. Careful autopsies revealed that in no instance was there any sign of hemorrhage at the base of the brain

|| Recent unpublished experiments show that the pituitary antiserum as well as P.U. antisera will inhibit the gonadotropic action of pregnant mare serum.