smears of the animals receiving their first injection during diestrus revealed that with 2 exceptions, they failed to present a fully cornified smear after injections were started; in several, however, there was a mixed smear of nucleated epithelium and cornified cells on the second or third day of the injection period, the normal time of the next expected estrus. After this initial attempt at estrus in some of the rats, diestrus was prevalent throughout the remainder of the injection period. These studies indicate that testosterone-propionate possesses the capacity to stimulate luteinization when injected in sufficient amounts and that the degree of luteinization obtained is greatest when injections are initiated in estrus or metestrus. Furthermore, the degree of mucification seems in our experiments to be correlated with the degree of luteinization induced.

Studies on the pituitaries of these rats are incomplete. In the glands studied the basophiles have been completely or almost completely degranulated. In most instances the eosinophiles also presented evidence of degranulation and in some of the glands the relative level of these cells was reduced. In these latter glands there was an increase in the percentages of the chromophobes; some were enlarged and possessed enlarged negative images of the Golgi apparatus. The changes in the eosinophiles and chromophobes noted in some of these rats are in contrast to previous findings in which in normal or castrated immature rats 10 daily injections of 500 gamma of male hormone induced degranulation of the basophiles but no noteworthy changes in the eosinophiles or chromophobes.[1, 2] It should be noted that in the experiments here reported we have used a dosage of 10 daily injections of 2000 gamma of male hormone and a different type of test animal.

9506

Some Properties of the Antigonadotropic Factor.

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While continuing our studies on the antigonadotropic factor of the blood[1] we examined the following properties:

1. *Thermolability.* The antigonadotropic factor was heated in a

[1] PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 708, 712.

water-bath and its effectiveness was tested in rats according to the technique indicated by us.[1] Table I shows the result of examination at pH 8.0.

TABLE I.
Thermolability of the antigonadotropic factor (pH = 8.0).

Preparation	Degree of Heating	Duration of Heating	Effectiveness
Serum	56°C	1 hr.	+
"	62°C	1 "	+
"	70°C	1 "	+
Diluted Serum	80°C	1 "	—
" "	100°C (boiling)	until the appearance of the first bubble	—
Acetone Dry Powder	100°C (water bath)	1 hr.	+

Inactivation at 80° and 100° was performed while diluting 1:4, in order to avoid coagulation of the serum. Reactivation of the serum by adding fresh rabbit blood (complement) failed. The antigonadotropic factor is not destroyed by heating for one hour at 70°C.; it is destroyed, however, if heated for one hour up to 80°C. as well as by boiling up once. An acetone dry powder preparation of the antigonadotropic factor is not destroyed by one hour's heating at 100°C.

2. *Influence of pH.* Fifty mg. acetone dry powder of an antigonadotropic serum were dissolved in 4.0 cc. n/10 NaOH (pH, 13) and allowed to stand in the refrigerator for 24 hours. Then we neutralized with some drops of n HCl and added prolan. Titration in an infantile rat showed that the prolan was able to produce the HVR (anterior pituitary reactions). Consequently the antigonadotropic factor had been destroyed. In a similar way we examined the effectiveness of more diluted lyes, n/50 NaOH (pH, 12.5) and n/50 NH_4OH (pH, 11). Here the antigonadotropic factor was no longer destroyed.

In a third series 50 mg. acetone dry powder of an antigonadotropic serum were dissolved in 4.0 n/10 HCl (pH, 1) and allowed to stand in the refrigerator for 24 hours. Then we neutralized with some drops of n NaOH and added prolan. In the rat test there was no prolan effect at all; consequently the antigonadotropic factor had not been destroyed.

n/10 HCl (pH, 1) and strongly diluted lyes: n/50 NaOH (pH, 12.5) or n/50 NH_4OH (pH, 11) do not affect the antigonadotropic factor. n/10 NaOH (pH, 13), however, destroys the antigonadotropic factor.

3. *Influence of pepsin digestion.* 100 mg. of pepsin were dis-

solved in 4.0 NaCl-HCl-buffer (pH, 2.0), and 70 mg. of acetone dry powder of an antigonadotropic serum added (50 PAU*). After 24 hours in the incubator the solution was alkalized with NaOH up to pH = 9.6 (indicator: thymol blue), in order to inhibit a further pepsin effect. Then prolan was added. In the rat test we found that the prolan produced the HVR. Consequently pepsin had destroyed the antigonadotropic factor. At the same time we showed in control experiments that our pepsin in an acid solution (pH, 2.0) destroyed prolan, that in an alkaline solution (pH, 9.6) it does not affect it.

The antigonadotropic factor is destroyed by pepsin.

4. *Influence of trypsin digestion.* 100 mg. of trypsin were dissolved in 3.0 NaCl-Na_2CO_3-buffer of pH = 9.5 and 50 mg. acetone dry powder of an antigonadotropic serum were added (50 PAU). After 24 hours in the incubator the solution was centrifugalized and acidified with HCl up to pH = 3.5 (indicator: bromphenol blue), in order to avoid a further trypsin effect. Then prolan was added. The rat test showed that the prolan produced the HVR. Consequently trypsin had destroyed the antigonadotropic factor. At the same time we indicated in control experiments that our trypsin in an alkaline solution (pH, 9.5) destroyed prolan, that in an acid solution (pH, 3.5) however, it does not affect it.

The antigonadotropic factor is destroyed by trypsin.

5. *Resistance of the antigonadotropic factor to ultraviolet irradiation.* 50 mg. acetone dry powder of an antigonadotropic serum (50 PAU) were dissolved in 4.5 cc. of distilled water, hermetically shut in a quartz vessel 5 mm. deep and exposed to the light of a quartz lamp at a distance of 20 cm. for 30 minutes while being constantly cooled by an electric fan. The temperature did not exceed 40°C. The pH was 8.0. Then prolan was added. In the rat test we found that the prolan did not produce the HVR.

Ultraviolet irradiation (30 minutes, 20 cm. distance) does not affect the antigonadotropic factor.

6. *Resistance of the antigonadotropic factor to oxidation.* 100 mg. acetone dry powder of an antigonadotropic serum (100 PAU) were dissolved in 10.0 cc. of a 1% hydrogen peroxide solution and allowed to stand at room temperature for 24 hours. Then, by the catalase effect of 10 drops of blood, the oxygen was liberated, in order to

* 1 PAU = 1 prolan anti-unit is the smallest amount of the antigonadotropic factor able to annihilate the gonadotropic effect of 1 RU of prolan. At least 10 units must be assayed in a test rat. (cf. Note 1.)

avoid action on prolan.[2] Prolan was added. The rat test revealed that the solution was still able to inactivate 100 RU of prolan. Thus the antigonadotropic factor had not been destroyed.

A 1% hydrogen peroxide solution does not affect the antigonadotropic factor.

7. *Solubility of the antigonadotropic factor in acetone.* We added to acetone in increasing concentration 25 mg. (25 PAU) respectively of dry powder of an antigonadotropic serum. Then the acetone was allowed to evaporate in the incubator for 24 hours and to the remainder distilled water was added.

Table II shows the conditions of solubility.

TABLE II.
Solubility of the Antigonadotropic Factor in Acetone.

Concentration of acetone %	Solubility of the antigonadotropic factor
10-40	easily soluble
45	slightly soluble
50-100	insoluble

The antigonadotropic factor is soluble in 40% acetone, insoluble in 50% acetone.

8. *Effect of the antigonadotropic factor upon a prolan solution masked by a 5% glycocoll solution.* It is well-known that we are able to inhibit the effect of some enzymatic substances by "masking" them with a 5% glycocoll solution. Thus we tried to protect 10 RU of prolan from the effect of the adequate amount of antigonadotropic factor by a contact of one hour with 2.5 cc. of a 5% glycocoll solution at a temperature of 37°C. The rat test revealed that no HVR (anterior pituitary reaction) occurred at all. Consequently the antigonadotropic factor exerts its effect upon the prolan-glycocoll complex in the same way as upon prolan. The reverse experiment failed as well: Antiprolan masked in a glycocoll solution maintained its effectiveness against prolan in the rat test.

The contact of prolan with a "masking" glycocoll solution does not protect it against the antagonistic effect of the antigonadotropic factor. Just the same glycocoll does not exert any influence upon the effectiveness of the antigonadotropic factor.

9. *Dialysis.* In order to ascertain the possibility of dialysis of the antigonadotropic factor we chose dialysis tubes of cellophane

[2] v. Euler, H., and Zondek, B., *Skand. Arch. Physiol.*, 1934, **68**, 232; Zondek, B., *Hormone d. Ovariums und d. Hypophysenvorderlappens*, Vienna, J. Springer, 2nd ed., 1935, p. 242.

and cuprophane. 100 mg. = 100 PAU of an antiprolan dry powder were dissolved in distilled water and dialysis was performed for 24 hours at room temperature against 50.0 cc. aqua test. The dialysate was then inspissated in the vacuum at a temperature not exceeding 30°C. from 50.0 cc. to 5.0 cc., mixed with 10 RU of prolan and titrated in the rat test. The rats went into oestrus. Consequently the dialysate contained less than 10 PAU. The fluid in the tube in its turn revealed no loss of titre.

The antigonadotropic factor does not dialyze through cellophane and cuprophane membranes.

The preceding studies reveal considerable differences between prolan and antiprolan. Prolan is almost completely destroyed by heating for one hour to 67°C. (up to 90%)[2]; the antigonadotropic factor, however, is destroyed only at 80°C. Prolan is strongly affected (according to Evans[3]) by n/10 HCl, but never by n/10 NaOH. The antigonadotropic factor, reversely, is destroyed by n/10 NaOH, not, however, by n/10 HCl. Pepsin and trypsin digest prolan and the antigonadotropic factor in the same way. Prolan is very sensitive to ultraviolet rays,[2] it is destroyed within 15 minutes at a distance of 20 cm.[2] The antigonadotropic factor, however, is not destroyed at this distance, even if exposed to the rays for 30 minutes. Prolan is destroyed in a great part by a 1% hydrogen peroxide solution, the antigonadotropic factor is not influenced by this substance. The behavior towards glycocoll solutions is rather similar for prolan and antiprolan. The solubility in acetone is about the same for prolan and antiprolan.

As to the solubility of the antigonadotropic substance in acetone, there is a considerable difference if compared with the antithyrotropic factor. Anderson and Collip[4] point out that the antithyrotropic substance can be dissolved in 66% acetone. We, however, find the antigonadotropic factor to be soluble only up to a 40% acetone. Other differences are as follows: Eitel and Loeser[5] indicate that the antithyrotropic protective substance is to be found not only in the blood but in the spleen and the liver of the preliminarily treated animals. This statement does not apply to our antigonadotropic principle. It is to be found exclusively in the blood but never in other organs, nor in the urine.[1] The latter writers, furthermore, aver that the formation of the antithyrotropic factor is dependent upon the presence of the thyroid gland, or at least, in thyroidecto-

[3] Evans, H. M., *Mem. Univ. Calif.*, 1933, **2**, 66.

[4] Anderson, E. M., and Collip, J. B., *Lancet*, 1934, 784.

[5] Loeser, A., *Arch. Exp. Path. u. Pharm.*, 1936, **184**, 23.

mized animals, upon the simultaneous effect of the thyroidal incretion (simultaneous application of thyroxin). We found the antigonadotropic substance in the blood of castrates as well.[1] The antithyrotropic principle circulates more or less in every organism, as Collip discovered. We performed similar examinations with the antigonadotropic hormone and found that a normal serum—in our rat test—was more likely to increase than to inhibit the effect of an added prolan solution. Collip's antithyrotropic principle does not react specifically as to the species. The antigonadotropic factor, however, examined by us reacts highly specifically as to the species, it has even, to a great degree, a relatively high organ-specificity.[1]

Summary. In the above experiments we pointed out the following properties of the antigonadotropic factor derived from blood: It is not destroyed by one hour's heating to 70°C. It is, however, destroyed at 80°C., and by being boiled up once in diluted solutions. Heated for one hour at 100°C. in an acetone dry powder preparation it will not be destroyed. It is destroyed by pepsin, trypsin and n/10 NaOH. It is, however not affected by n/50 NaOH, n/50 NH_4OH, n/10 HCl, 1% H_2O_2-solution, ultraviolet irradiation. It is soluble in 40% acetone, insoluble in 50% acetone. It does not dialyze through cellophane and cuprophane membranes. We cannot exert any influence upon the mechanism of the prolan-antiprolan effect by "masking" with 5% glycocoll solutions. We pointed out the difference between the gonadotropic and the antigonadotropic factor, as well as between the antigonadotropic and antithyrotropic factor.

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Mechanism of Prolan-Antiprolan-Reaction in Simultaneous and Unsimultaneous Application of Both Active Principles.

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In previous investigations on antiprolan[1, 2] we used a certain standard method for determining the antigonadotropic effect. We chose the simultaneous injection of prolan and antiprolan after 2

1 Zondek and Sulman, PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 708.
2 *Ibid.*, 1937, **36**, 712.