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.

9507

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

BERNHARD ZONDEK AND FELIX SULMAN.

From the Laboratory, Obstetrical-Gynecological Department, Rothschild-Hadassah Hospital, Jerusalem.

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.

hours' incubation. In the meantime we varied the time factor and investigated which differences of time could be permitted between the injection of prolan and that of antiprolan. We did not at first consider the quantitative side of the experiment, but compared qualitatively the effectiveness of large doses of antiprolan upon small doses of prolan with regard to the time within which an effect could be obtained.*

The experimental technique was as follows: Infantile female rats, 3-4 weeks of age weighing about 25-30 gm. received one subcutaneous injection each of 10 or 20 RU of prolan in the left flank. Besides this 10 times as great an amount of antiprolan (100-200 PAU†) was injected either previously, subsequently or simultaneously into the neck. Sixty hours after the first prolan injection we began with the vaginal smears and 60 hours later the animals were killed and uteri and ovaries examined macroscopically. Dubious cases were examined histologically in a complete series. First we determined the smallest amount of antigonadotropic factor which, after 2 hours' contact with 10 RU of prolan in the incubator, was able to annihilate the gonadotropic effect in the infantile rat. According to our above definition,* we called this minimal amount 10 PAU.

1. In a first series we injected 10 PAU of antiprolan into the neck of infantile rats simultaneously with 10 RU of prolan into the flank. The gonadotropic effect was only incompletely impaired, *i. e.*, there remained a certain excess of prolan, sufficient to bring about proestrus in the animal and to induce a slight increase in weight of ovaries. The intraperitoneal injection also proved to be inferior to the "incubator-contact method." If we have a certain antiprolan excess, however, the separate injection of antiprolan and prolan can be used with as great a certainty as the simultaneous injection of a mixture of prolan and antiprolan after 2 hours' contact in the incubator.

2. In the following series of experiments antiprolan was injected previously to prolan and the result of the gonadotropic reaction determined (Table I).

Table I demonstrates that antiprolan is still effective 8 days after injection into the body. During this time the rats are refractory toward 10 RU of prolan. After 10 days, however, the effect of antiprolan can no longer be proved.

* Quantitative results will be reported later.

† 1 PAU = 1 prolan anti-unit is the smallest amount of antiprolan able to annihilate the gonadotropic effect of 1 RU of prolan. At least 10 units must be assayed in a test rat.

TABLE I.
Result of the Gonadotropic Reaction After Previous Injection of Antiprolan.

Antiprolan Injected First PAU	Prolan Injected Later RU	Difference of Time	Gonadotropic Reaction HVR I, II, III
100	10	1 hr.	neg.
100	10	6 "	"
200	20	12 "	"
200	20	1 day	"
200	20	2 "	"
200	20	3 "	"
100	10	6 "	"
100	10	8 "	"
100	10	10 "	pos.

We investigated further as to whether antiprolan circulates in the blood while it is still effective or is deposited in the organs. We injected 100 PAU of antiprolan subcutaneously into young rats weighing 20 gm. Sixty hours later the animal was bled completely and the circulatory system washed, starting from the heart, with 5 cc. of normal saline. Then the lavage water and the blood serum (on an average a total of 6 cc.) were injected into infantile female rats together with 10 RU of prolan. The gonadotropic reaction was negative. These results furnish proof for the assumption that the antiprolan circulates in the blood even as long as 60 hours after injection.

We examined the bodies of rats preliminarily treated with antiprolan for their content of this factor, using the extraction technique indicated by us previously.[1] We found that if the bleeding was insufficient, from the 100 PAU of antiprolan which had been injected 10 PAU could be proved even 60 hours after injection in the body of the animal. If the bleeding was sufficient even that 10% of antiprolan could not be traced. The small quantities of antiprolan which can occasionally be found in the body are probably attached to remnants of blood which remain in the organs when the bleeding is insufficient. In any case the separate extraction of the organ fractions (muscle, bones, central nervous system on the one hand and skin on the other) did not reveal any quantities of antiprolan.

3. In the third series inversely prolan was injected previously to antiprolan and the results of the gonadotropic reaction were then remarked (Table II).

Table II shows that even 12 hours after the injection of prolan we assuredly succeed in annihilating the gonadotropic reactions by means of antiprolan. Twenty-four hours later in one part of the experiment the prolan effect was completely annihilated, in another

TABLE II.
Result of the Gonadotropic Reaction in Subsequent Injection of Antiprolan.

Prolan Injected First RU	Antiprolan Injected Later PAU	Difference of Time	Gonadotropic Reaction HVR I, II, III
10	100	10 min.	neg.
10	100	30 "	"
20	200	1 hr.	"
20	200	2 "	"
20	200	6 "	"
20	200	12 "	"
10	100	24 "	"
20	200	24 "	Slight swelling of the follicles and proestrus
10	100	36 "	pos.

part we found a slight prolan effect (slight swelling of the follicles and proestrus). This shows that 24 hours is exactly the space of time during which we can succeed in annihilating the gonadotropic effect by means of antiprolan.

4. In a further series we investigated whether gonadotropic reaction could be inhibited by way of the digestive tract, while separately applying prolan and antiprolan, *i. e.*, we administered antiprolan enterally. One hundred and fifty PAU of antiprolan were prepared as an acetone dry powder (60 mg.) and dissolved in 1 cc, of distilled water. One half was then given to an infantile female rat per oesophageal sound (human urethral catheter). Three hours later the second portion was given per sound. One hour after that 10 RU of prolan were injected subcutaneously. The gonadotropic reaction was positive, which means that antiprolan has no effect when applied enterally. This is in conformity with our observation[2] that antiprolan is destroyed by pepsin and trypsin. Consequently, the effect of antiprolan can be produced only in the parenteral way.

The above experiments tell us something about the mechanism of the effect of antiprolan. The peculiarities with regard to species and organ specificity of antiprolan mentioned previously[2] had given an indication that we should not have to deal here with a purely hormonal mechanism. We said at that time that this mechanism certainly did not run humorally, as *e. g.*, the insulin-adrenalin-mechanism controlling the sugar metabolism, but that the 2 active principles (prolan and antiprolan) must have a relation to one another like that of a key to the lock to which it belongs. If we could succeed in preserving the effect of antiprolan in the body for 8 days, *i. e.*, in rendering the body of the rat insusceptible to prolan, it would mean also that antiprolan does not work like a hormone, but rather as an antibody, by the help of which we are enabled to exert passive immuniza-

tion for a certain period. As was shown by one of us previously[3] the antigonadotropic factor, even though it cannot be recognized by means of the usual methods for proving antibodies (precipitation and complement fixation), is very similar to the antibodies in many respects.

The fact that we succeeded in the rat in annihilating the gonadotropic effect by means of the homologous antigonadotropic factor even 24 hours after application of prolan seems to be contrary to the results of Parkes and Rowlands.[4] They succeed only one hour after cohabitation in inhibiting rupture of the follicle by the injection of heterologous antigonadotropic factor in the rabbit. The authors used an antigonadotropic serum prepared by prolonged injection of rabbits with *ox* anterior pituitary extract. If the authors had had a homologous antigonadotropic factor (against rabbit pituitaries) at their disposal, it is highly probable that they would have been successful in inhibiting the follicular rupture even after more than an hour. Besides this the gonadotropic effect is more quickly produced in rabbits than in rats. This probably explains the difference in our results.

Moreover, the experiments show that the prolan effect in the ovary of the rat is not an immediate, but a protracted one, and in the beginning still preventable. The stimulating effect of prolan A (follicle ripening hormone) upon the follicle produces irreversible changes only after 24 hours, which now proceed automatically and unavoidably lead to estrus.

Summary. (1) Antiprolan has a paralyzing effect upon prolan in separate as well as in simultaneous injection in the test rat. Nevertheless, the inhibiting effect of antiprolan is stronger if we inject a prolan-antiprolan mixture which has been allowed to stand in the incubator for 2 hours. (2) The antigonadotropic effect can be observed 8 days after the application of antiprolan (100 PAU). During this period the test animal is refractory towards prolan (10 RU). (3) Antiprolan circulates in the blood of the test rat as long as 60 hours after subcutaneous injection. It cannot be proved in the organs of the rats at that time. (4) We are able to retard the gonadotropic effect of prolan in rats up to 24 hours after the injection, if we inject antiprolan within this interval. (5) If applied enterally antiprolan has no effect whatsoever.

[3] Sulman, F., *J. Exp. Med.*, 1937, **65**, 1.

[4] Parkes and Rowlands, *J. Physiol.*, 1936, **88**, 305.