Castrated male and female rabbits formed the antigonadotropic factor in their blood in the same manner as normal animals.

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The Antigonadotropic Factor. Species Specificity and Organ Specificity.

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There are contradictions to be found in literature as to the specificity of the antigonadotropic factor. Bachmann, Collip and Selye,[1] Fluhmann,[2] Meyer and Gustus,[3] Brandt and Goldhammer,[4] Twombly,[5] Sulman,[6] Thompson,[7] hold that the species specificity of the antigonadotropic factor is proved. Gegerson, Clark and Kurzrok,[8] Rowlands,[9] Parkes and Rowlands,[10] however, presented evidence against the species specificity. Recently Collip[11] reported similarly. However, the latter workers had other test objects (inhibition of ovulation in the mated rabbit or of the oestral cycle in the normal rat) than the former,[1-7] and than we did. Fluhmann,[2] Brandt and Goldhammer,[4] Gegerson, Clark and Kurzrok,[8] Parkes and Rowlands[10] are opposed to the presence of an organ specificity. Selye, Collip and Thompson,[12] however, favor it. To investigate this we performed the following experiments. We used the technique described in our first report,[13] that of the exact titration of the gonadotropic hormone against adequate amounts of the antigonadotropic factor in infantile female rats and mice.

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1 Collip and Selye, PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 544.

2 Fluhmann, PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1595; *Am. J. Obst. Gynec.*, 1935, **30**, 584.

3 Meyer and Gustus, *Science*, 1935, **81**, 208.

4 Brandt and Goldhammer, *Z. f. Immunitaetsforschung*, 1936, **88**, 79.

5 Twombly, *Endocrinology*, 1936, **20**, 311.

6 Sulman, *J. Exp. Med.*, 1937, **65**, 1.

7 Thompson, PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 634.

8 Gegerson, Clark and Kurzrok, PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 193.

9 Rowlands, *Proc. Roy. Soc. London*, 1937, B, No. 824, **121**, 517.

10 Parkes and Rowlands, *J. Physiol.*, 1936, **88**, 305; *Lancet*, 1937, p. 924.

11 Collip, *Canad. Med. Assoc. J.*, 1937, **36**, 199.

12 Selye, Collip and Thompson, PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 487, 566.

13 Zondek and Sulman, PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 708.

While studying the species specificity and organ specificity it was essential to pay attention to the minimal and maximal values. We, therefore, thoroughly titrated maximal amounts of the antigonadotropic factor (50-100-200 PAU or PSAU*) against minimal amounts of such gonadotropic factor foreign to the species or to the organ, (1-5-10 RU). In control experiments we confirmed the titre of the gonadotropic and that of the antigonadotropic factor. The results are shown in Table I.

Table I shows (Experiments 1 and 4) that the antigonadotropic sera are active against the gonadotropic preparation used for the preliminary treatment. In contrast to this 200 PSAU (Experiment No. 6) cannot inactivate one RU of gonadotropic hormone from another species, *i. e.*, an antigonadotropic serum has less than 0.5% of its effectiveness if used against a heterologous gonadotropic factor.

In further experimental series (Table II) we studied the organ specificity of human gonadotropic hormone derived from a different starting material (urine, blood, pituitary). In Table II we find unmistakably the explanation of the extent of the organ specificity of the antigonadotropic factor. The effectiveness of the pregnancy-urine-prolan "antiserum" against the homologous "antigen" is demonstrated in Exp. 1. Exps. 2 and 4 show that the effectiveness of this "antiserum" against blood prolan or pituitary prosylan of the same species amounts to only about 7% of its titre against pregnancy-urine-prolan; *i. e.*, compared with the strict species specificity there is only a relatively high organ specificity. It was only by using highly concentrated "antisera" and by titrating exactly ascertained large doses of the antigonadotropic factor against such doses of gonadotropic substance which were certainly effective and exactly titrated that we arrived at these results.

The following experiment represents a further indication as to the species specificity of the antigonadotropic factor. Four female rats got 100 RU of prolan twice weekly subcutaneously during a period of over a year. In 2 of the animals the antigonadotropic titre of the blood was examined. Because of the non-intense preliminary treatment (only twice a week) it was low (20 PAU per cc.). In the

* 1PAU = prolan anti-unit is the minutest amount of the antigonadotropic factor required to annihilate the gon. effect of 1 RU prolan in the immature female rat. 1PSAU = 1 prosylan anti-unit is similarly defined. N.B. At least 10 units should be assayed in a test rat. We call prolan the gonadotropic hormone derived from pregnancy urine, prosylan that containing, in addition to prolan, synprolan (synergetic factor), *cf.* Zondek, B., *Act. Obst. et Gyn. Scandin.*, 1935, **15**, 1.

TABLE I.
Species Specificity of the Antigonadotropic Factor.

Exp. No.	Antigonadotropic factor against	Anti-units PAU-PSAU	Gonadotropic factor from	Rat Units RU	HVR I-III (gonadotropic reaction)	Result: antigonadotropic effect
						%
1.	human pregnancy urine prolan†	100 PAU	human pregnancy urine prolan†	100 RU	neg.	100
2.	,, ,,	100 PAU	pregnant mare's serum prosylan†	5 RU	pos.	< 5
3.	,, ,,	100 PAU	bovine hypophyseal prosylan†	5 RU	,,	< 5
4.	pregnant mare's serum prosylan†	200 PSAU	pregnant mare's serum prosylan†	200 RU	neg.	100
5.	,, ,,	200 PSAU	human pregnancy urine prolan†	5 RU	pos.	< 2.5
6.	,, ,,	200 PSAU	bovine hypophyseal prosylan†	1 RU	,,	< 0.5

TABLE II.
Organ Specificity of the Antigonadotropic Factor.

Exp. No.	Antigonadotropic factor against	Anti-units PAU	Gonadotropic factor from	Rat Units RU	HVR I-III (gonadotropic reaction)	Result: antigonadotropic effect
						%
1.	human pregnancy urine prolan†	100	human pregnancy urine prolan†	100 RU	neg.	100
2.	same	100	human pregnancy blood prolan‡	7 RU	,,	> 7
3.	,,	100	same	15 RU	pos.	< 15
4.	,,	100	human antepituitary prosylan‡	7 RU	neg.	> 7
5.	,,	100	same	15 RU	pos.	< 15

‡ The pregnancy-blood-prolan was prepared as an acetone dry powder, the hypophysis prosylan according to the method of Van Dyke and Wallen-Lawrence.

other 2 animals one ovary respectively was extirpated. Each of them averaged 14 mg. in weight and in the histological examination they showed a few moderately enlarged follicles as well as some old vascularized corpora lutea. The ovaries, therefore, revealed only a weak reaction upon prolonged prolan treatment. But when we injected 100 MU of antex† (pregnant mare's serum prosylan) the remaining ovary reacted with an increase in weight up to 7 times the amount (100 mg.) and with enormous swelling of the follicles, blood spot formation and luteinization. (HVR I, II and III.) This experiment also suggests the species specific behavior of the antigonadotropic factor: the ovaries did not react further with gonadotropic hormone of human origin, they did, however, react strongly with hormone of animal origin.

No doubt in every organism not preliminarily treated there are in addition to these specific antigonadotropic factors, non-specific gonadotropic antagonists which may be looked upon as antihormones in the strictest sense of the word. According to Evans, *et al.*, such an antagonist is to be found in the pituitary,[14] according to Engelin in the epiphysis,[15] and according to Hoffmann, *et al.*, in the adrenals.[16] Prompted by the results of these workers we performed the following experiments:

We injected into an infantile female rat an aqueous extract of 200 mg. acetone dry powder from bovine epiphyses. The following day the injection was repeated and 10 RU of prolan added. The gonadotropic reaction (HVR) as usual was read 120 hours later. No inhibition of the gonadotropic reaction could be found. We repeated the experiments with 2 ampules of Epiphysan§ which had the same negative result. There are, consequently, no observable amounts of gonadotropic antihormone in the epiphysis.

We injected into an infantile rat 1.0 Eschatin‖ (adrenal cortex extract) and on the following day 0.5 Eschatin + 10 RU of prolan. A similar experiment was started with half the dose of Eschatin. The result after 120 hours was in 2 of the experiments complete

† We used prolan from pregnancy urine of the I. G Farben-Ind.; antex, from pregnant mare's serum of the Løvens Kemiske, Kopenhagen; preglandol, from bovine pituitaries of Hoffmann-La Roche, Basel. We are indebted to the above firms for kindly supplying the preparations.

[14] Evans, Korpi, Pencharz and Simpson, *Univ. Calif. Publ. Anat.*, 1936, **1**, 237.

[15] Engelin, *Klin. Wschr.*, 1935, **14**, 970; *Wien. Klin. Wschr.*, 1935, **48**, 1160.

[16] Hoffmann *et al.*, *Klin. Wschr.*, 1937, **16**, 79.

§ We are indebted to the firm Gideon Richter, Budapest, for kindly supplying the preparations.

‖ We are indebted to the firm Parke, Davis & Co. for kindly supplying the preparations.

inhibition of the gonadotropic reaction; in 4 further experiments, however, no such inhibition was found. We repeated the experiment with the same doses of Cortigen§ and did not find inhibition of the gonadotropic reaction. The adrenals, therefore, do not contain noticeable amounts of antigonadotropic hormone.

We hold that the above experiments explain the contradiction in literature as to the species specificity and organ specificity of the antigonadotropic factor. These contradictions are based upon the fact that the majority of the workers approached the problem from the qualitative side, not from the quantitative one. Those workers[9-11] who are opposed to the species specificity inhibited the normal oestral cycle in the rat or the ovulation in the mated rabbit, etc., by applying comparatively enormous doses of the antigonadotropic factor. The rat, to bring about its oestrous phase, produces only about one RU of gonadotropic factor, consequently the experiments of the workers[9-11] only prove that large amounts of antigonadotropic factor (especially if applied daily) are able to annihilate the effect of one RU of heterologous (foreign to the species) prosylan. Without doubting the correctness of these investigations we approached the question quantitatively, and found that antigonadotropic sera show less than 0.5% of their effectiveness against a heterologous gonadotropic factor, involving a loss of titre by at least 99.5%. Even if the conclusions drawn by the workers[9-11] from their experiments are fully correct as to their theoretical meaning (that there is no absolute species specificity) our quantitative experiments reveal practically that the species specificity is enormous (more than 99.5%). On these grounds we think we may be permitted to state: The antigonadotropic factor works specifically to the species.

Regarding the organ specificity we draw the conclusion: The antigonadotropic factor against human pregnancy-urine-prolan reveals only 7% of its titre if used against human pregnancy-blood-prolan or against prolan of human pituitary origin, *i. e.*, a loss of 93% of its effectiveness.

So in a serological sense every prolan preparation has a different antigenic structure. Another difference between the gonadotropic preparations derived from different sources is variation in time necessary for the formation of the antigonadotropic factor. Thus prolan derived from human pregnancy urine induces the formation of antiprolan in the rabbit after one month's treatment; gonadotropic hormone derived, however, from human or mare's pregnancy blood requires about 3 times as long. We believe the quantitative method of investigation proposed by us to be suitable for giving a closer

insight into the difference in structure of the gonadotropic factors derived from different sources.

The property of a strict species specificity and of a relatively high organ specificity of the antigonadotropic factor gives us a clue as to the mechanism of the antigonadotropic effect. It is certainly not a humoral antagonistic effect (as *e. g.*, to be found in the insulin-adrenalin-contra-mechanism) but a process having its effective point directly and specifically in the gonadotropic preparation and rendering the preparation itself ineffective biochemically. We shall furthermore report experiments leading to the supposition that the gonadotropic hormone irreversibly breaks down and is destroyed by the homologous antigonadotropic factor.

Basing our deductions upon the above investigations we have to differentiate between 2 groups of gonadotropic antihormones:

First Group. Antigonadotropic factors formed in the blood following protracted treatment with a heterologous gonadotropic hormone (preferably daily subcutaneous or intravenous application). The formation of these antigonadotropic factors is exclusively connected with the blood, independent of the species of animal, sex and the presence of sexual functions (castration). These antigonadotropic factors are likely to take their effect by splitting up the gonadotropic hormone. They reveal less than 0.5% of their effectiveness if used against a heterologous gonadotropic factor, 7% of their effectiveness if used against a homologous gonadotropic factor, which is, however, foreign to the organ.

Second Group. Gonadotropic antagonists of the normal non-treated organism. Such antagonists are said to occur in the pituitary and in the adrenals of normal animals not having been preliminarily treated. The mechanism of their effectiveness is hormonal, unspecific to species and organ. Their recognition in the pituitary gland and in the adrenals is rendered difficult because these organs produce simultaneously several hormones. Thus does the pituitary liberate hormones inducing the gonadotropic reaction and the adrenals such hormones advancing it. In the epiphysis we could not find any noticeable amounts of the gonadotropic antagonistic factor.