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# Does the Antigonadotropic Factor Occur in the Organism under Physiological and Pathological Conditions?

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The question as to whether the antigonadotropic factor is present in the organism of humans as well as animals under physiological conditions is one of fundamental importance. On this depends the question whether the formation of the so-called anti-hormones should be considered a regulatory mechanism (in accordance with Collip's definition) working in the organism under normal conditions, or whether it is a protective reaction somewhat like the immunity reaction.

We attempted to prove the presence of antigonadotropic substances in organisms which had not been subjected to preliminary treatment with gonadotropic hormone. We had the choice of two technics: (1) The serological method *(in vitro)*, and (2) the gonadotropic anterior pituitary reactions[1] *(in vivo)*. The negative results yielded by the use of the serological method have already been reported.[2]

In the following paragraphs we are going to describe our animal experiments.

1. *Does the antigonadotropic factor occur in the blood of patients who suffer from ovarian insufficiency?* Collip[3] reported the occurrence of antigonadotropic substances in the blood of women who had not been subjected to preliminary treatment. Laroche and Simmonet[4] found the antigonadotropic factor (antiprolan) in the blood of one castrata and one oligomenorrhoic woman. We tried to corroborate these findings, examining, moreover, women who suffered from primary amenorrhoea, secondary amenorrhoea or sterility. Our results were, however, negative. We analyzed quantities of 1-9 cc of serum against 1-5 RU of prolan. In the above mentioned cases not even 1 PAU* could be detected in 9 cc of serum.

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1 Zondek, B., and Aschheim, S., *Klin. Wsch.*, 1927, No. 6.

2 Sulman, F., *J. Exp. Med.*, 1937, **65**, 1.

3 Collip, J. B., *J. Mt. Sinai Hosp.*, 1934, **1**, 28; *Ann. Int. Med.*, 1935, **9**, 50.

4 Laroche and Simmonet, *Compt. rend. Soc. Biol.*, 1936, **121**, 416.

* 1 PAU = 1 prolan anti-unit is the smallest amount of the antigonadotropic factor required to annihilate the gonadotropic effect (estrous reaction) of 1 RU prolan in the immature female rat (*cf.* Zondek, B., and Sulman, F., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 708).

2. *Does the antigonadotropic factor occur in the blood of patients suffering from tumors?* Flaks and Ber[5] furnished data on the occurrence of antigonadotropic substances in the urine of patients suffering from tumors. We investigated the occurrence of antigonadotropic substances in the serum of carcinomatous and sarcomatous patients. The sera assayed contained nothing of the antigonadotropic substance (less than 1 PAU per 3 cc of serum).

3. *Does the antigonadotropic factor occur during pregnancy?* It is a well established fact that the nidation of the ovum, in primates, is accompanied by a sudden rise of the proportion of prolan in the organism.[6] The prolan concentration then gradually diminishes until the end of pregnancy. Nature thus provides us with an example of a physiological flooding of the organism with large amounts of prolan, and the continuous influx of this substance should provide an ideal condition for the formation of antiprolan. The progressive regular decrease of the amount of prolan with advancing pregnancy should be explained (as according to Collip's antihormone-buffer-theory) by the formation of antiprolan.

It is not without technical difficulty that the presence of antiprolan is demonstrated, especially as the large quantities of prolan which are still present in the blood obscure the activity of antiprolan. The estrone content of the pregnancy serum also interferes with the verification of the presence of antiprolan, if the procedure is carried out with the estrus method (prolan A). A special experimental set-up is, therefore, required. We may use one of the following methods:

(A) The human pregnancy blood may be examined during puerperium after prolan as well as estrone have disappeared from the blood. Experiments set up in this way showed that there was no antiprolan in the blood of the puerpera one week after delivery. Fluhmann[7] has reported similar results.

(B) The pregnancy blood may be examined in such animals (or the foeti of these animals) in whom, normally, the production of prolan during pregnancy does not reach measurable proportions, which may be due to the production of antiprolan. Thus the experiments were carried out with rats, but neither in the mother nor in the foetus could antiprolan be detected at the time of delivery.

(C) As the most useful method we had to consider the analysis of pregnancy blood according to the technic indicated by us[8] (disso-

5 Flaks, J., and Ber, A., *Dwutygodnika Medycyna* (Poland), No. 2, 1939.

6 Aschheim, S., and Zondek, B., *Klin. Wschr.*, 1928, No. 30, 31.

7 Fluhmann, C. F., *Tr. Am. Gyn. Soc.*, 1935, **60**, 237.

8 Zondek, B., and Sulman, F., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 343.

ciation of the prolan-antiprolan mixture). If a prolan-antiprolan mixture is subjected to ultraviolet irradiation or to the action of $H_2O_2$ or HCl, prolan is destroyed[9] while antiprolan is not affected[10] and can be proved by means of the usual method (inactivation of freshly added prolan[8]). Ultraviolet irradiation and $H_2O_2$ did not, however, completely destroy the prolan so that this method was not practicable either. Neither was the experiment with HCl fully convincing as to its results, since the concentration of HCl necessary to destroy prolan was such as could equally destroy antiprolan. If a medium is used which contains little protein and electrolyte buffer it is, indeed, possible to destroy the urine-prolan with n/10 to n/15 HCl (during 20 hours at room temperature) without the antiprolan (from serum) which has been added being affected. In the serum prolan, a medium which contains much protein, quantitative destruction of prolan B by means of n/10 to n/15 HCl did not, however, succeed; n/5 HCl had to be used (pH = 1.1 at 37°C for 20 hours) in order completely to destroy the prolan in the serum. But in this way antiprolan was also destroyed. We, therefore, abandoned this method and chose the more successful procedure of dissociating pregnancy serum with NaOH.[8] We did not use non-treated pregnancy serum but prepared an acetone-dry-powder (according to the technic described[2]) from the fluids we wanted to assay: pregnancy blood, pregnancy urine, retroplacental blood, placental blood, amniotic fluid, puerperium blood. These types of dry-powder contain no estrone, while the concentration of prolan and antiprolan is practically the same as that of the serum (60-80 mg are equivalent to 1 cc of serum). If kept in the exsiccator these powders maintain their titers with reference to prolan or antiprolan for years, which greatly facilitates repeated tests and titrations even for the minutest amounts of prolan or antiprolan. Addition of NaOH to this powder very easily destroys antiprolan, while prolan is not affected, provided that the adequate concentration is adhered to.

We started from the hypothesis that our acetone-dry-powders prepared from the various fluids of the pregnant human organism must necessarily contain antiprolan masked by prolan. We, therefore, arranged 15 experimental series in which NaOH in varying concentrations (from n/5 to n/50) was added to the acetone-dry-powders. Any amount of antiprolan present in the powder would have necessarily been destroyed by NaOH, thus increasing the

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

[10] Zondek, B., and Sulman, F., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 193.

gonadotropic titers of the dry-powders. The experimental set-up was the following:

*First experimental series: titration of prolan.* Increasing doses of acetone-dry-powder from pregnancy blood, etc., were dissolved in n/10 NaCl and kept at room temperature for 24 hours, after 2 drops of brom-thymol blue had been added in order to check up the pH concentration. Then the neutral solutions were injected into infantile female rats, in 6 doses over a period of 36 hours, and in this way the prolan A titer was determined (estrus reaction).

*Second experimental series: proof of antiprolan.* Corresponding amounts of serum-dry-powder were dissolved in NaOH of varying concentrations (n/5 to n/50) in order to destroy any antiprolan which might happen to be present. They were also kept at room temperature for 24 hours after addition of brom-thymol blue. After this, the alkaline solution was neutralized by adding some drops of normal HCl and the gonadotropic titer determined according to the method mentioned above. As long as n/50 to n/15 NaOH was used no deviation of the gonadotropic titer was observed. The gonadotropic titer decreased, however, as soon as n/10 to n/5 NaOH was used, since the increasing NaOH concentration destroyed the prolan. With no NaOH dilution we did, however, achieve an increased prolan titer of our dry-powders, as should have been expected if antiprolan were present.

*Summary.* We may conclude from the results of the above experiments that no antiprolan is produced in the human organism during pregnancy. This has been ascertained in connection with the blood during the first as well as the last months of pregnancy, during delivery and one week postpartum. It is, therefore, evident that the organism does not use the antigonadotropic factor as a regulatory mechanism under physiological conditions. Antiprolan is exclusively elaborated in the organism after a type of prolan foreign to the species has been administered. Antiprolan formation must, therefore, be considered as a protective mechanism on pathological lines, as is similarly found in the immunity reactions, but not as a regulatory measure with reference to endogenous hormonal conditions. In accordance with this, no antiprolan has been proved to be present either in individual organs or in the blood of normal, amenorrhoic or sterile women or patients suffering from tumors.