Zeitschrift: Bulletin der Schweizerischen Akademie der Medizinischen Wissenschaften = Bulletin de l'Académie suisse des sciences médicales = Bollettino dell' Accademia svizzera delle scienze mediche

Herausgeber: Schweizerische Akademie der Medizinischen Wissenschaften

Band: 25 (1969)

Artikel: Central effects of endogenous progesterone and synthetic progestational agents

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DOI: https://doi.org/10.5169/seals-307787

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Central Effects of Endogenous Progesterone and Synthetic Progestational Agents

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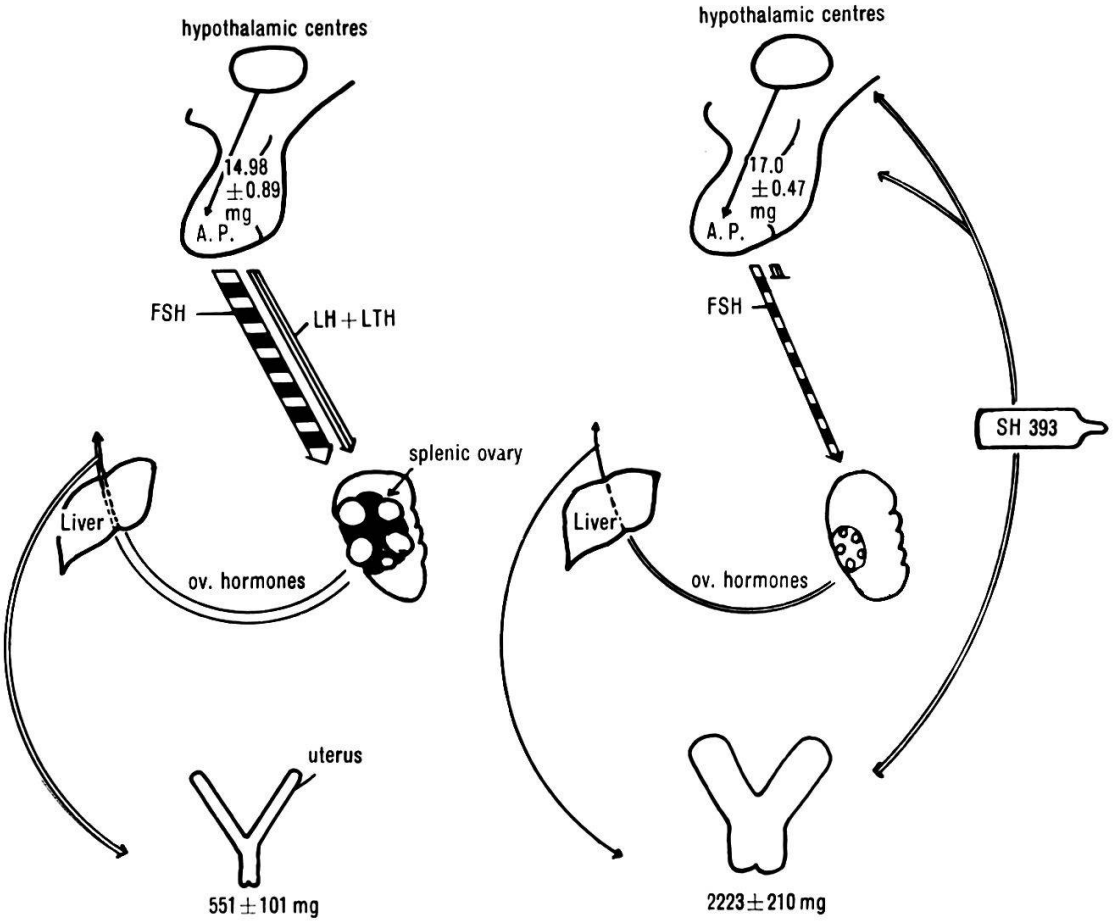
The central effects of progestational synthetic agents can be studied in animal experiments and by means of clinical observations. Though animal experiments cannot be transferred to human beings without criticism, we have to keep in mind that they may contribute to our pool of information which is necessary for an exact clinical application of hormonal steroids. The interest in the mode of action and duration of action of depot-injections of different steroidal agents has been increasing recently. We have developed a special test in guinea pigs, the "splenic ovarian graft planimetric test" which permits us to study 1. the central anti-gonadotropic and the peripheral uterotropic effects of hormonal steroids, 2. the duration of action of one depot-injection, and 3. the reversibility of ovulation inhibition. Among others the following compounds have been studied: norethisterone-enanthate, norethisterone-acetate, and hydroxy-norprogesteronecapronate.

Fig. 1 shows the principle of the test in a diagram. Virginal guinea pigs of 300–400 g are castrated, and one ovary is implanted into the spleen. The intralienal graft is vascularized in the spleen and produces hormones which go via the portal circulation to the liver. Here the hormones are inactivated. The uninhibited anterior pituitary gland and hypothalamic centres now release higher quantities of gonadotropic hormones which stimulate the intrasplenic graft, so that it hypertrophies. The different structures of the intralienal graft are studied in serial sections. The maximal cystic follicular, haemorrhagic follicular, and luteinized areas are measured planimetrically as indicator of the gonadotropic activity.

If norethisterone-enanthate is given, as it is symbolized in the right diagram, the hypertrophy of the intralienal graft is prevented, and no luteal body formation is observed after 90 days of treatment. LH is totally inhibited, and the release of FSH is partially suppressed. The peripheral uterotropic effect of norethisterone-enanthate can be demonstrated by the increase of weight of the uterus. The average uterine weight of the norethisterone-enanthate-treated animals is four times as high as that of the control animals, symbolized on the left side of the diagram. Clinically the uterotropic effect may be utilized in inducing a pseudo-pregnancy.

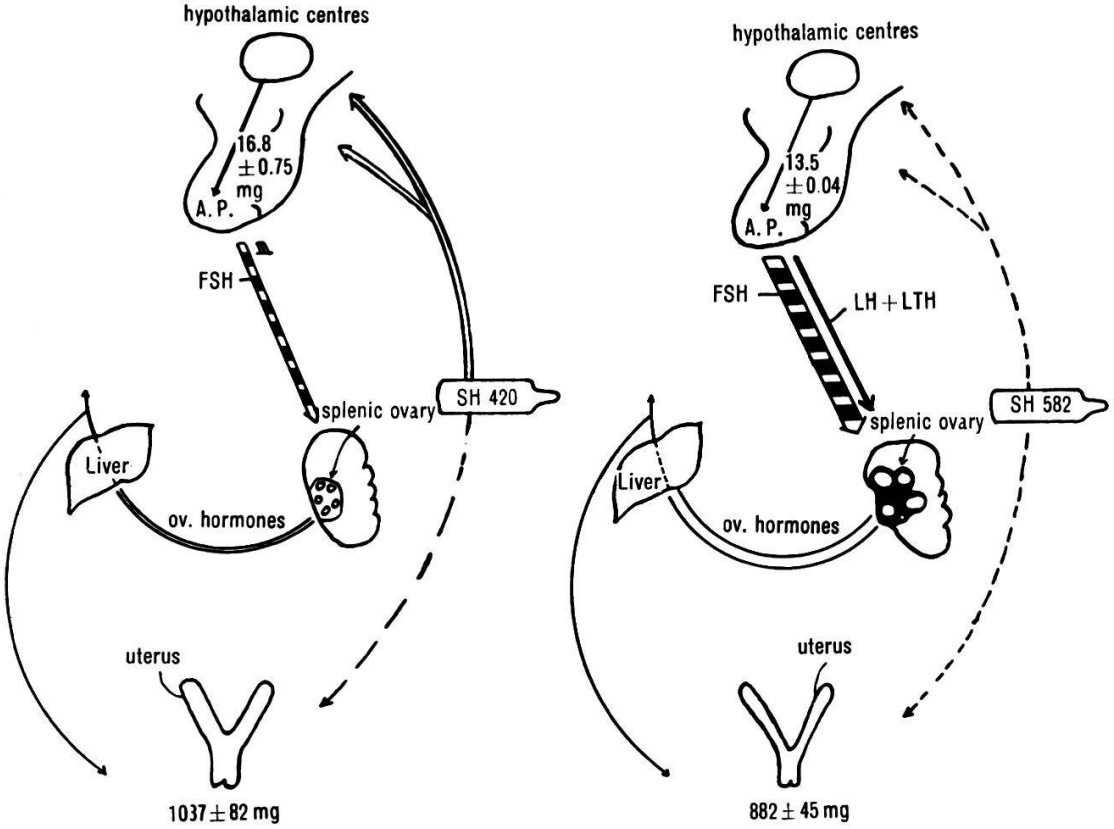


1 Nonius unit (N.U.) = 9765 μ^2

Average cystic follicle area	458 N.U.	697 N.U.		169 N.U.	174 N.U.
Average hemorrhagic follicle area	239 N.U.			4.5 N.U.	
Average luteinized area	209 N.U.			0 N.U.	

Fig. 1. Testing of norethisterone-enanthate in the splenic ovarian graft planimetric test. Method: exstirpation of one ovary and implantation of the other one in the spleen. – Left: untreated guinea pigs. Hypertrophy of splenic ovarian graft. – Right: guinea pigs treated with norethisterone-enanthate. Prevention of luteal body formation and of cystic ovaries. Strong uterotropic effect.

The diagram on the left side (Fig. 2) illustrates the results of treatment with norethisterone-acetate, and on the right side of another series of animals treated with hydroxy-norprogesteronecapronate. Norethisterone-acetate develops – like norethisterone-enanthate – a strong central gonadotropine inhibiting effect insofar as the intrasplenic ovarian grafts do not show any luteal body formation and a strong reduction of cystic follicular areas. FSH is partially inhibited, and the release and/or secretion of LH/LTH is totally blocked. The peripheral uterotropic effect is not as strong as that of norethisterone-enanthate. The average uterine weight, however, is still twice as high as that of the control animals. In contrast to these findings hydroxy-norprogesteronecapronate reduces only slightly the luteinized areas of the intralienal grafts, whereas the average cystic and haemorrhagic



1 Nonius unit (N.U.) = 9765 μ^2

Average cystic follicle area	256 N.U.	256 N.U.	611 N.U.	885 N.U.
Average hemorrhagic follicle area	0 N.U.		274 N.U.	
Average luteinized area	0 N.U.		168 N.U.	

Fig. 2. Testing of norethisterone-acetate (left diagram) and hydroxy-norprogesterone-capronate (right diagram) in the splenic ovarian graft planimetric test. Norethisterone-acetate (SH 420) shows a strong anti-gonadotropic effect with blockade of LH/LTH and a reduction in the release of FSH. Hydroxy-norprogesteronecapronate (SH 582) exerts no effect on FSH release and only a mild effect on LH/LTH secretion. SH 582, therefore, has only a very weak central effect and no uterotropic effect.

follicular areas are not reduced. This may be interpreted as a partial inhibition of LH or LTH, whereas the FSH secretion is not altered. The central effect of hydroxy-norprogesteronecapronate is, therefore, relatively small, and solely confined to an alteration of LH or LTH. There is also scarcely any peripheral uterotropic effect.

Fig. 3 gives an idea of the exact size of the different planimetrically measured structures of the intralienal grafts. The two upper circles are representing the control animals. The circle on the left shows the status of an ovary at the time of implantation in the spleen. It can be seen that after 90 days of implantation into the spleen (circle on the right), the planimetrically measured white maximal cystic follicular arease and the hatched maximal luteinized areas have increased in size, and we find also haemorrhagic follicles, the average maximal areas of which are shown in black. In the lower row of circles another series of animals has been treated with

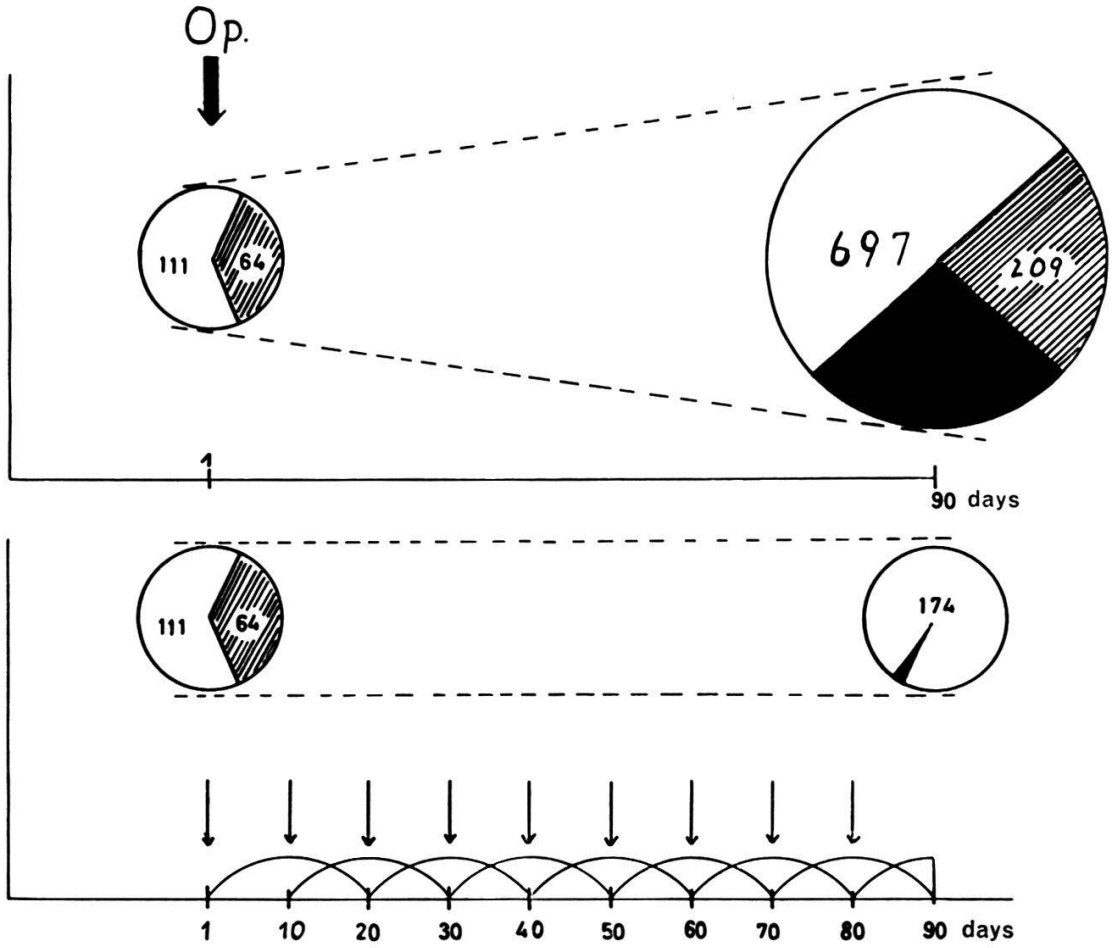


Fig. 3. Prolonged treatment with norethisterone-enanthate; 10 mg as depot-injection every 10 days. Upper row of circles: untreated animals. Lower row of circles: treated animals. White: average maximal cystic follicular areas. Black: average maximal planimetrically measured haemorrhagic follicular areas. Hatched: planimetrically measured maximal luteinized areas. Every circle demonstrates the results with a series of 30 animals. The numbers represent the planimetrically measured Nonius units (1 N.U. = 9765 μ^2).

norethisteroneenanthate over 90 days; that means 10 mg as depot-injection every 10 days. The effect of successive injections is overlapping and results in a rather continuous inhibition of gonadotropic hormones which prevents the hypertrophy of the intralienal graft. The different numbers are representing Nonius-units, 1 N.U. corresponding to 9765 μ^2.

Fig. 4 shows a serial section of a guinea pig ovary before implantation in the spleen. Fig. 5 demonstrates the hypertrophy of the intralienal graft after 90 days of implantation in the spleen with luteal body formation and the development of cystic and haemorrhagic follicles. Fig. 6 shows that under treatment with norethisterone-enanthate the hypertrophy of the intralienal graft is prevented. Fig. 7 shows a typical size of the uterus of a control animal after 90 days of implantation of the ovary in the spleen: Fig. 8 demonstrates the uterine hypertrophy after 90 days of treatment with norethisterone-enanthate indicating the strong uterotropic effect of this progestational agent.

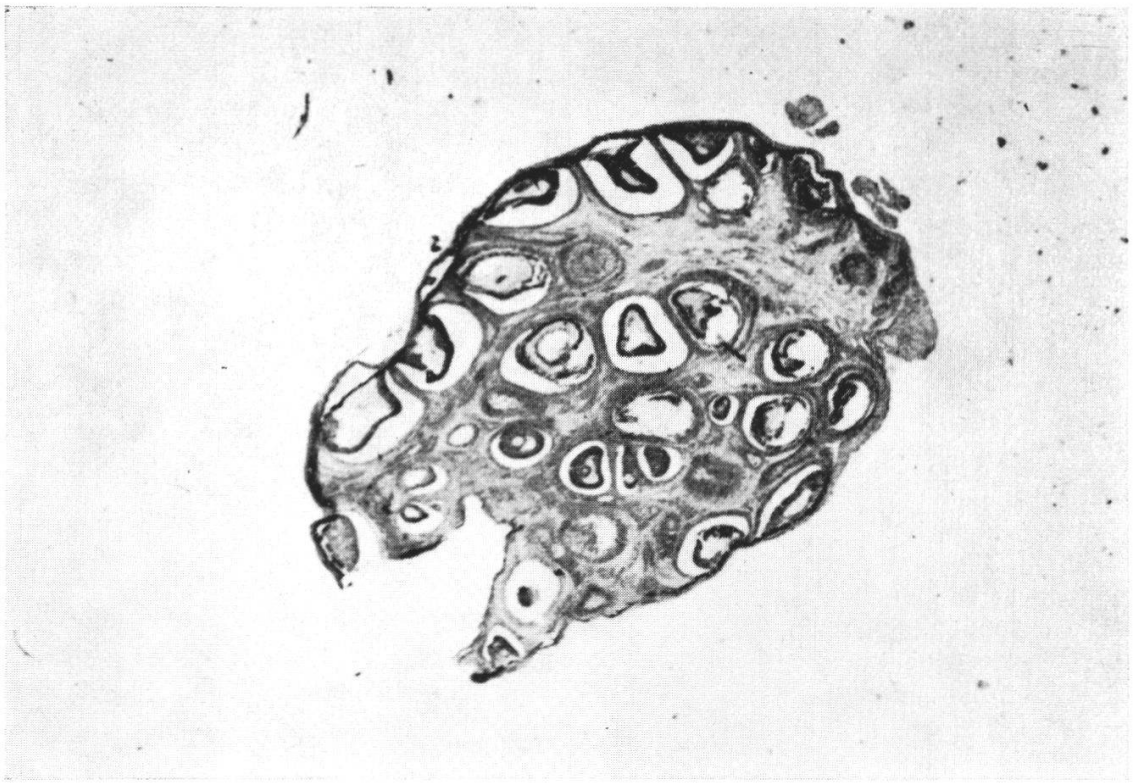


Fig. 4. Serial section of an ovary of a guinea pig before implantation into the spleen.

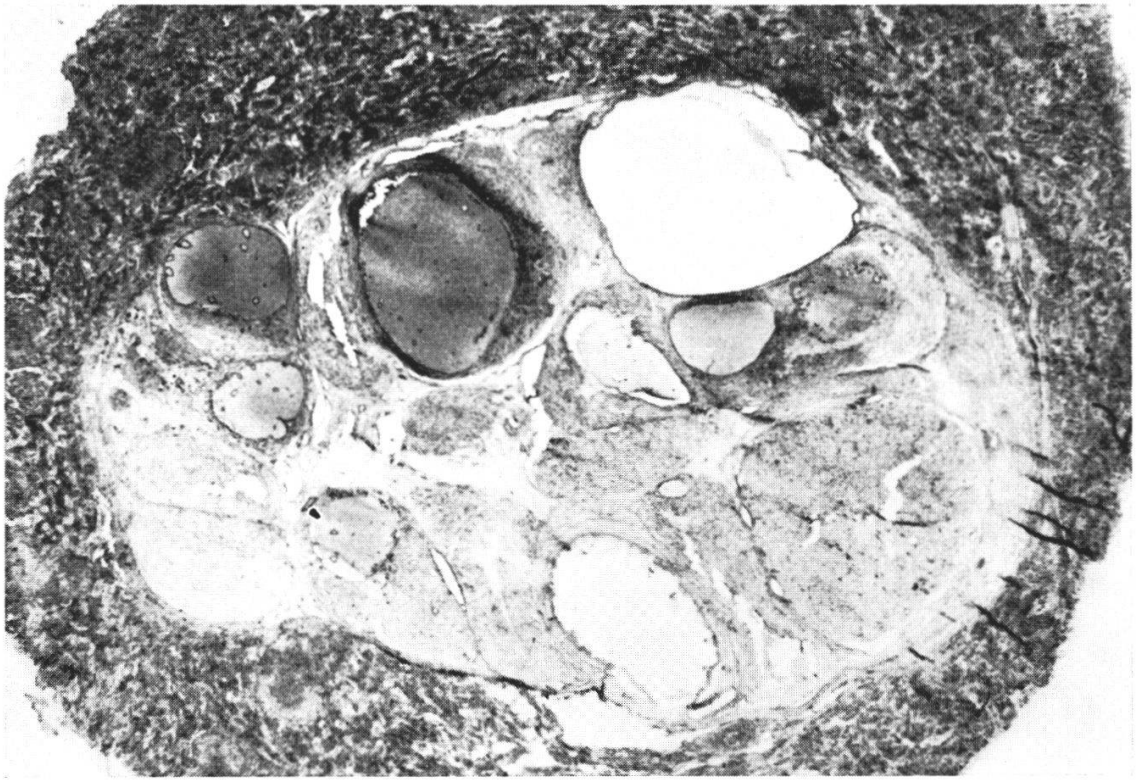


Fig. 5. Hypertrophy of intralienal ovarian graft after 90 days of implantation in the spleen. The other ovary had been exstirpated. Untreated control. Formation of luteal bodies and great cystic and haemorrhagic follicles.

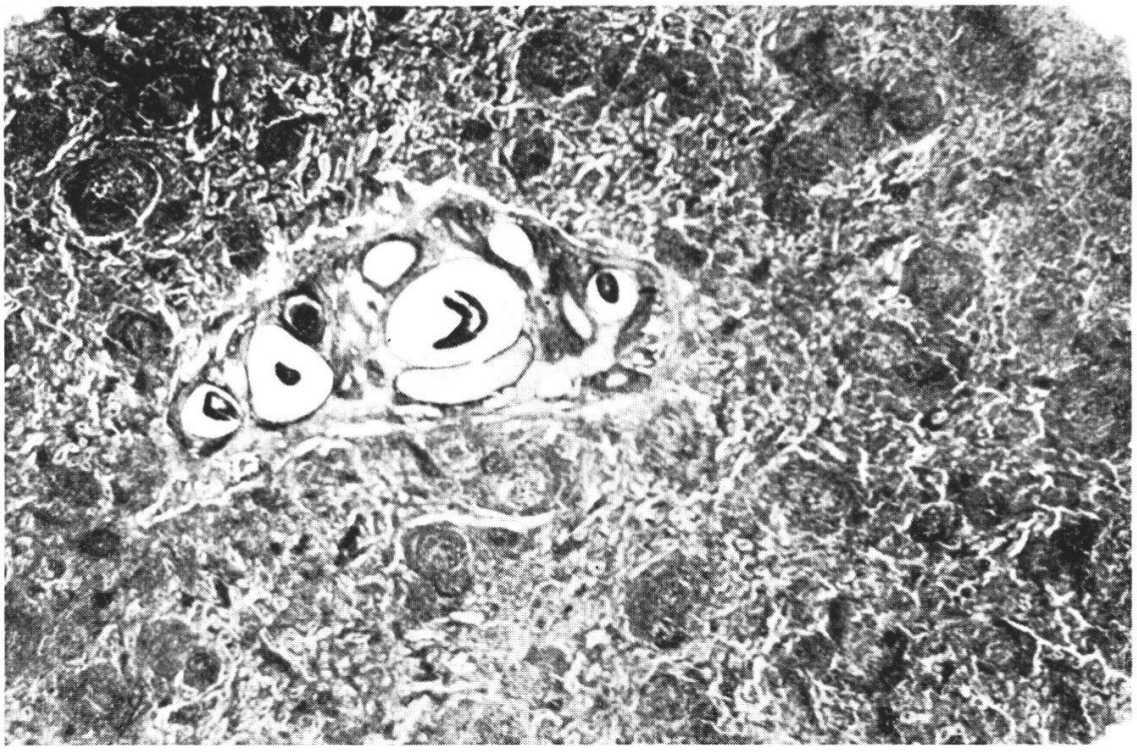


Fig. 6. Prevention of hypertrophy of intralienal graft and prevention of luteal body formation as well as of cystic follicular development under treatment with norethisterone-enanthate.

The duration of the antigonadotropic effect of one injection of 20 mg of norethisterone-enanthate has been studied in a modification of this splenic ovarian planimetric test (Fig. 9). The upper row of circles again shows the different follicular and luteinized areas in different series of untreated control animals after 57 and 70 days of implantation time in the spleen. If we inject 20 mg of norethisterone-enanthate on the 50th day of the experiment and sacrifice different series of animals on the 57th, 64th, and 70th day after the operation, it can be seen, in the lower row of circles, that there is already a certain reduction in the follicular areas 2 weeks after the injection, whereas 3 weeks after the injection the follicular areas already increase again, compared with the untreated control animals. The effect on FSH is, therefore, maximal after 2 weeks and wears off after 3 weeks. If we consider the hatched segments of the circle, we observe after 7 days no effect on the luteinized areas but after 2 weeks a definite reduction of the luteinized areas which persists and can be demonstrated even 3 weeks after the depot-injection. Fig. 10 illustrates the effect of norethisterone-enanthate on FSH in the left diagram and on LH/LTH in the right diagram. The black line corresponds to the untreated, the interrupted line to the treated animals. The cystic and haemorrhagic follicular areas, measured in Nonius-units on the left side, show a strong reduction after 2 weeks, whereas 3 weeks after the injection the difference to the control animals is decreasing. The effect on LH/LTH, shown in the luteinized areas on the right side, gives no difference in the luteinized areas after 7 days, but an increasing difference

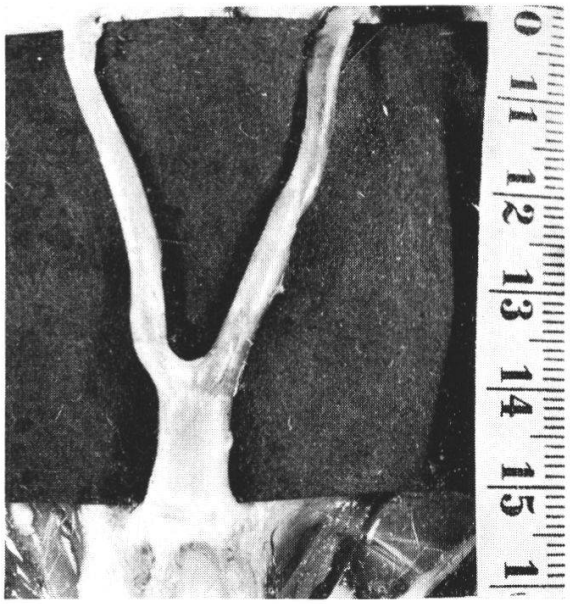


Fig. 7. Uterus of control animal. 90 days after one ovary was exstirpated and the other ovary implanted in the spleen.

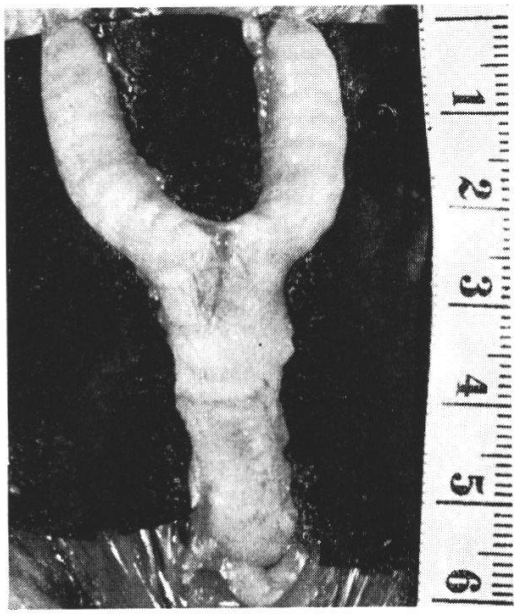


Fig. 8. Operation in the same way as animal of Fig. 7: treatment with 90 mg norethisterone-enanthate, 10 mg every 10 days over 90 days. Strong uterotropic effect of norethisterone-enanthate.

with prolonged reduction of the luteinized areas even 3 weeks after the injection.

Fig. 11 shows another modification of the splenic ovarian graft planimetric test which permits us to study the *reversibility of the antigonadotropic effect.* The upper row of circles again demonstrates the increase in planimetrically measured follicular and luteinized areas after 90 and 120 days of implantation time in the spleen. In the lower row of circles we see that after 90 days of treatment with norethisterone-acetate, 10 mg every 10 days, the hyper-

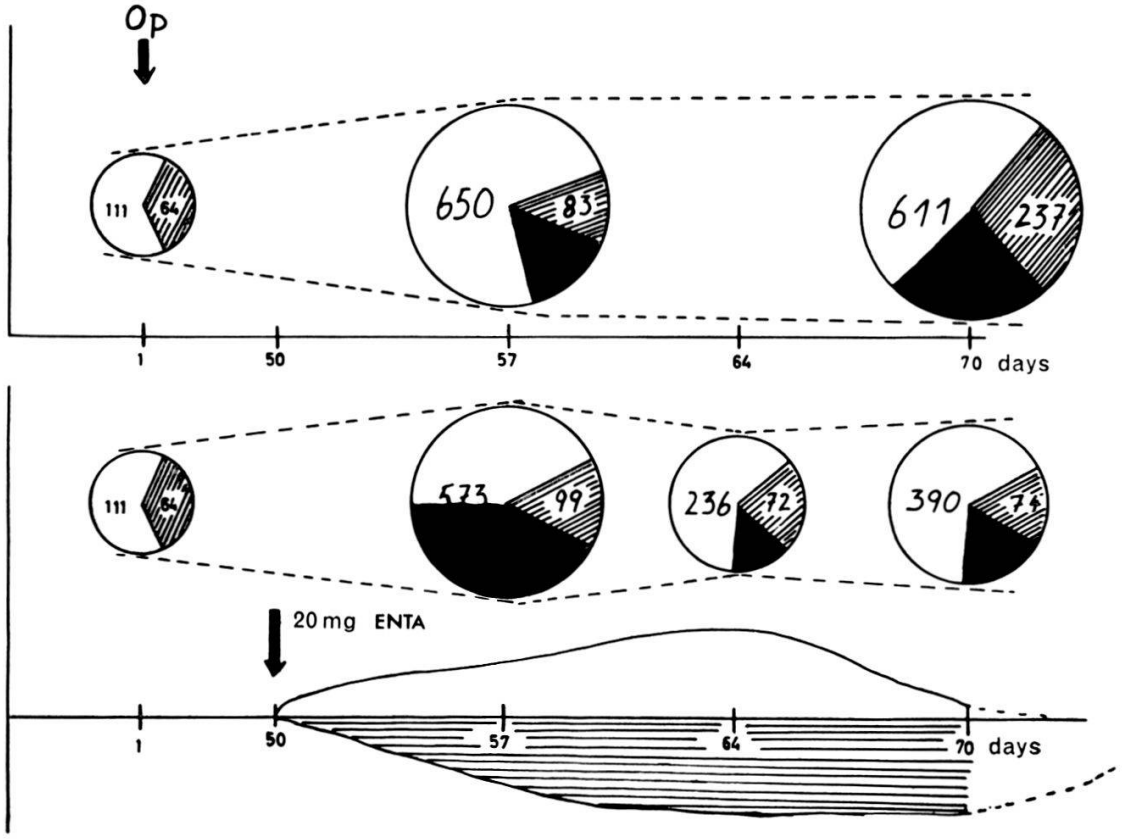


Fig. 9. Variation of the splenic ovarian graft planimetric test: testing of the central effect of one depot-injection of 20 mg norethisterone-enanthate. Planimetrically measured average maximal cystic follicular areas (white), haemorrhagic follicular areas (black), and luteinized areas (hatched) of control animals (upper row of circles) 57 and 70 days post operationem. Lower row of circles: injection of 20 mg norethisterone-enanthate (ENTA) on the 50th day after the implantation of the ovary in the spleen. Animals were sacrificed on the 57th, 64th, and 70th day after operation, that means 1, 2 respectively 3 weeks after the injection. One circle represents the average planimetrically measured follicular and luteinized areas of a series of 30 animals.

trophy of the intralienal graft has been prevented, and no luteal body formation can be noticed. Two other series of animals were treated in the same way up to the 90th day with norethisterone-acetate and were sacrificed on the 120th day and on the 200th day after the operation. It can be seen that 30 or 110 days after the end of the hormone treatment again an intensive hypertrophy of the intralienal graft has taken place. This can be demonstrated by the enormous increase in the luteinized areas (cross-hatched), cystic follicular areas (white), and haemorrhagic follicular areas (black). This indicates that the gonadotropin inhibition has been fully reversible and has even resulted in a strong rebound effect.

The central effect of 6-chloro-9β,10α-pregna-1,4,6-triene-3,20-dione (Ro 4-8347) has been studied in three unmarried women with proven ovulatory cycles and dysmenorrhoea. The volunteers were between 23 and 26 years of age. Normal ovarian function was ascertained by means of pregnanediol and gonadotropin excretion studies combined with the measurement of basal

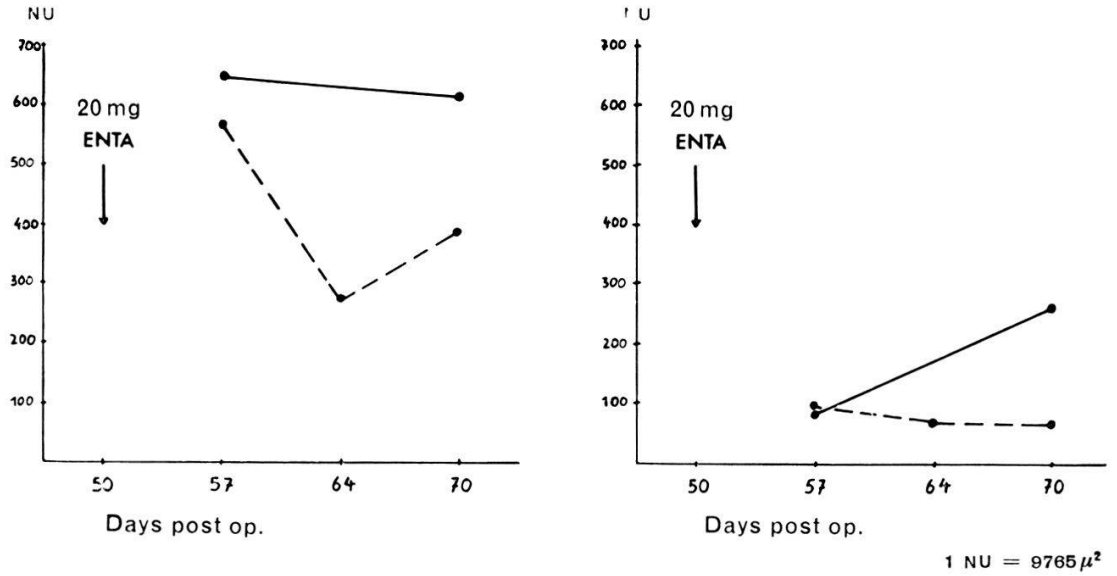


Fig. 10. Follicular areas (left diagram) and luteinized areas (right diagram) in Nonius units of control animals (black line) and series of animals treated with norethisterone-enanthate (ENTA) (20 mg as depot-injection) on the 50th postoperative day. The inhibition of FSH activity is strongest 2 weeks after the injection, whereas the LH/LTH inhibition persists undiminished for 3 weeks.

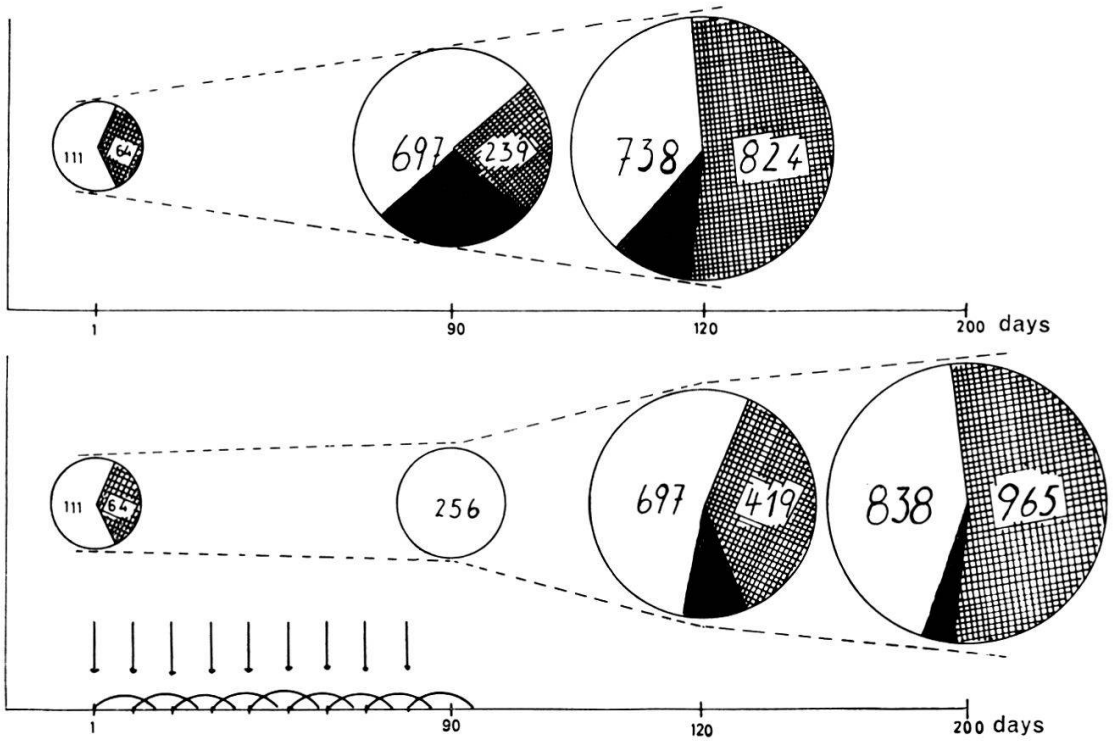


Fig. 11. Testing of reversibility of gestagen-induced gonadotropin inhibition after treatment with norethisterone-acetate (SH 420). Upper row of circle demonstrates the follicular and luteinized areas of untreated control animals 90 and 120 days after castration and implantation of one ovary in the spleen. Lower row of circles: treatment with 10 mg norethisterone-acetate at 10 days intervals up to the 90th day after the operation: inhibition of intrasplenic ovarian graft hypertrophy. Two other series of animals, treated in the same way, but sacrificed on the 120th and 200th day after the operation (that means 30 respectively 110 days after the end of hormonal treatment) proves the reversibility of the gestagen-induced gonadotropin inhibition: a genuine rebound effect.

body temperature and Papanicolaou smears in the first and second half of the cycle. In the first observation cycle no treatment was given. In the next four cycles 8, 6, 4, or 2 mg of Ro 4-8347 were administered daily from day 5 to 24 of the cycle. Two out of these three patients demonstrated an inhibition of ovulation when 8 mg of the retroprogesterone was administered daily, whereas 6, 4, and 2 mg did not prevent ovulation. The third patient, however, did not demonstrate any ovulation inhibition by any of the applied daily dosages. Under 8 mg we found, however, a very high stimulation of gonadotropin excretion which started already on the 8th ovulatory day and which at that time could not be correctly interpreted. (The hormone excretion studies were performed by Prof. ANNEMARIE KÖNIG, gynecological-endocrinological hormonal research laboratory of the women's hospital of the University of Göttingen.) The beneficial effect on the dysmenorrhoea of the three volunteers did not appear to be dependent on the inhibition of ovulation, but could also be observed when smaller daily dosages were given. We concluded from our observations that at about 8 mg per day we have achieved a hormonal level which in some patients is sufficient to inhibit ovulation. In smaller dosages apparently stimulation of gonadotropic activity may occur. We were, however, not lucky in stimulating ovulations in patients with anovulatory cycles, because we stopped this treatment when several patients reacted with a withdrawal bleeding after the application of 4–6 mg of the retroprogesterone daily from day 5 to 14 of the cycle.

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Discussion

B. LUNENFELD: I just have a methodical question to ask you. We are quite used to total cell counts in ovaries, and I am quite aware of the difficulties lying in the subject. However, could you just elaborate on how you perform the planimetric study? Are the ovaries cut into serial sections?

J. HALLER: Yes. Every series representing one circle is representing 30 animals. These animals are either control animals or treated in the same way. The intralienal graft is prepared and then you do serial sections. You get from every one of the intralienal grafts about 100 serial sections. First, you draw the outlines of the different ovarian structures – with the help of a drawing apparatus attached to a microscope – on a piece of paper and afterwards you measure planimetrically the different areas. I determined first that slide which shows the greatest luteinised area and then that indicating the greatest follicular area and afterwards that representing the greatest hemorrhagic follicular area. These representatives of all 30 animals are added together to determine the average planimetrically measured cystic follicular, hemorrhagic follicular respectively the average luteinised area of one series of animals. Thus, one average value shown in the segments of the circles which are used in the above illustrations is calculated from 3000 serial sections of the same series of animals. It is a quite tedious job and it takes some time.

B. LUNENFELD: Is there any mathematical evidence that such treatment of results is permissible?

J. HALLER: The series of animals have to be great enough to give representative data. According to our experience 20–30 identically treated animals per one series are sufficient for statistical evaluation. We have to be aware of the fact that this test is a semiquantitative biological assay which lasts over 90–200 days and, therefore, creates rather constant endocrinological functional relations which lead to rather gross significant anatomical differences of ovarian structure. The gross differences in the prolonged reversibility studies give one of the rare quantitative evidences proving the actual reversibility of the central antigonadotropic effect of certain steroids in the sense of a genuine rebound phenomenon.