Medicine. — *Biological Properties of Aethinyl-testosterone* [1]). By L. A. M. STOLTE. (From the Department of Pharmacology, University of Leiden. Director Prof. Dr. S. E. DE JONGH.) (Communicated by Prof. J. V. D. HOEVE.)

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I. *Progesterone-like properties.*

In 1938 INHOFFEN and HOHLWEG (1) prepared a substance, that may be called aethinyl-testosterone, pregneninolone or anhydro-oxyprogesterone, according to the view one has upon the structural formula. The most remarkable property of the substance is its progesterone-like activity upon the endometrium of the immature rabbit not only after subcutaneous but *also* after *oral* administration. Progesterone being orally inactive, the new substance immediately gained importance for the gynaecological praxis. In the course of 1939—1940 [2]) we investigated, how far aethinyl-testosterone can be considered to be biologically identical with progesterone. We further compared the quantities required of both substances, in order to obtain a certain effect. Aethinyl-testosterone indeed proved to possess a number of properties that may be called progesterone-like. Besides, we could show (and in view of the chemical nature of the substance, this was not so very surprising), that it bears testosterone-like properties and finally it proved unexpectedly to exert certain oestrogenic activities.

Other publications have appeared already in literature, in agreement with our findings: RUZICKA c.s. (2) and EMMENS & PARKES (3) also found a progesterone-like effect upon the endometrium of the immature rabbit.

COURRIER & JOST (4) and EMMENS & PARKES described a testosterone-like influence upon the cock's comb and upon the sex accessories in the male rat.

The former investigators could maintain the pregnancy of castrated rabbits with subcutaneous injections of aeth.-test. in high dosages. The latter authors found that aeth.-test. after subcutaneous as well as after oral administration produces oestrus in castrated rats.

We intend to describe in 2 papers 1. the progesterone-like and 2. the testosterone-like and oestrone-like properties of aeth.-test.

A few times aeth.-test. was administered in oily solution (max. 1 mgm pro 3 ccm); mostentimes it was emulgated in 1 ccm water with 200 mgm gummi arabicum. For the oral administration a stomach tube was used.

a. CLAUBERG-*test.* Our research pertaining to the relation, as reported by INHOFFEN and HOHLWEG ($^2/_3$ mgm progesterone subcutaneously ∞ 2 mgm

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[1]) For further details and literature vid. diss. Leiden 1940.

[2]) Organon Ltd generously supplied us with aethinyl-testosterone.

aeth.-test. subc. ∞ 4 mgm aeth.-test. orally = 1 K E) was confined to only some experiments.

In as much as the small number of animals allowed any conclusions, we got the impression that in our experiments the relation was 1 : 4 : 8—10 instead of 1 : 3 : 6.

3 mgm aeth.-test. (subcut. in emulsion) caused a ++/+++ effect in one rabbit; for the same result 6—8 mgm aeth.-test. (orally in emulsion) were required. Conversely $^2/_3$ mgm progesterone subcut., already caused a +++ effect.

Administration in oily solution or in an alcohol-water suspension of aeth.-test. (as used by INHOFFEN c.s.) gave no better results.

In order to allow a comparison, the effect of 10 mgm test. propr. and of 20 mgm testosterone was investigated. The result was resp. +/++ and O/+. Herewith it had become improbable that the progesterone-like activity of aeth.-test. upon the endometrium is due to the testosterone-like properties (KLEIN and PARKES) (5) of this substance.

In our further experiments we based ourselves upon the following relation:

progesterone subc. : aeth.-test. subc. : aeth.-test. orally 1 : 4 : 8—10.

b. Formation of deciduomata.

The experiment was done as described by SHELESNYAK (6): 16 immature rats (ca 3 weeks of age) were first treated with 2.5 γ oestrone during 4 days daily in 2 injections of 0.1 cc oil each.

The animals then were divided over 4 groups and received resp. 0.2 mgm progesterone subcut., 1 mgm aethinyl-testosterone subcut., 2.5 mgm aeth.-test. orally and 0.25 mgm testosterone subcut., daily for 7 days in 2 dosages a day.

The 8th day after the beginning of the experiment a silk thread was drawn through one of the uterine horns in longitudinal direction. Autopsy the 12th day.

Macroscopically all hurted horns proved to be unevenly thickened over the total length; distinctly isolated tumors could not be shown.

Microscopically circumscript tumor-like structures, consisting in decidual cells, were found in the endometrium of the animals, treated with progesterone and in these only. Though in the other groups a scattered decidual reaction existed (but in the intact horns as well!), it could be shown by staining with Gieson that connective tissue was involved in the process, whereas in the real deciduomata in the progesterone animals no such tissue could be detected. Conclusively, it is possible to make deciduomata appear with the aid of crystalline progesterone in immature rats, that underwent a precursory treatment with oestrone.

Neither the 4-fold quantity of aethinyl-testosterone (subcut.), nor the 10 fold quantity of aeth.-test. (orally), nor finally the equal quantity of testosterone (subcut.) proved to be able to effect the same.

On the base of dates in literature, concerning oestrone and testosterone in combination with progesterone, this failure is possibly due to the oestrogenic properties of aethinyl-testosterone still to be described. (Oestrone + progesterone, vid. COURRIER (7), SELYE and MC KEOWN (8)). On the other hand a disturbing influence of the testosterone-like component must be considered improbable (testosterone + progesterone, BROOKSBY (9)).

c. Influence upon the sensibility of the rabbit uterus to oxytocine.

The influence upon the uterine motility was researched upon 3 castrated rabbits with abdominal window.

Rabbit I received 100 int. U oestrone daily during 12 days. With 0.01—0.001 Voegtlin U of Piton [1]) a distinct increase (when compared with "spontaneous") of the total duration of the uterine contractions could be obtained.

After the subcutaneous administration of daily 100 int. U of oestrone, combined with 4 mgm aeth.-test. orally, the threshold of the sensibility to Piton went up to 1—0.1 V U. A 9 days treatment with solely oestrone was followed by a decrease to 0.001 V U.

In rabbit II the threshold was 0.01—0.001 V U Piton after a treatment with 100 int. U oestrone daily during 10 days. After 100 int. U oestrone + 16 mgm aeth.-test. (subcut.) on 5 subsequent days it had become 10 V U; after a 11 days treatment with oestrone only, it went down as far as to 0.001—0.0001 V U.

The combination of the oestrone with 0.4 mgm progesterone made the threshold rise to 0.01—0.001 V U in 6 days.

In rabbit III a threshold of 0.001 V U was reached after 7 subsequent daily injections of 100 int. U oestrone. After 5 following days of 100 int. U oestrone + 10 mgm aeth.-test. (orally) the threshold increased to 1—0.1 V U. After 100 int. U oestrone during 9 days it had diminished to 0.1—0.01 V U; after 100 int. U oestrone, combined with 5 mgm aeth.-test. (subcut.) it ascended to 1—0.1 V U.

This showed, that aeth.-test., subcutaneously administered as well as orally, is able to lower the sensitiveness of the rabbit uterus to oxytocine. However, the uterine contractions make a very uncoordinate impression, when under the influence of aeth.-test. The uterus has a "spotty" appearance and the contractions don't proceed regularly over the surface of the organ, as is the case under the influence of progesterone. Furthermore it was observed that under the influence of aeth.-test. the spontaneous motility decreases only little or not at all. This too is contrarious to what has been reported in literature (VAN WOERKOM (10), REYNOLDS c.s. (11)) on progesterone.

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[1]) "Piton" (Organon) is an oxytocically active preparation manufactured from the posterior pituitary lobe.

d. Maintenance of Pregnancy after castration.

1. Pregnant *rats* were bilaterally castrated on the 5th, 10th or 14th day after sperm had been shown in the vaginal smear. To begin with the day before castration the animals were treated with resp. 1.25 mgm progesterone, 5 mgm aeth.-test. (subcut.) and 10 mgm aeth.-test. orally, all in 2 portions daily unto the 18th—20th day, whereupon autopsy was performed on the following day in order to establish wether the fetusses were present and if so to count the number of the living and the dead ones. A control group was solely treated with gummi arabicum.

TABLE I.

Day of castration	Daily dosage			
	G. arab.	1.25 mgm Prog.	5 mgm aeth. test. subc.	10 mgm aeth. test. orally
5th	4 rats −, −, −, −	3 rats +, +, −	3 rats −, −, −	3 rats −, −, −
10th	3 rats −, −, −	5 rats ++, ++, +, −	5 rats −, −, −, −, −,	 −, −, −
14th	3 rats ++, +, +	3 rats ++, ++, ++	3 rats ++, +, −	 ++, +, +

++ Living fetusses.
\+ Dead fetusses or placent. remainders.
− absence of any remainder.

As the table (I) shows, the effect of aeth.-test. orally or subcutaneously administered, is inferior by far to that of progesterone in view of the "maintenance" of pregnancy, at least when the substances are given in the aforementioned quantitative relation of 8 : 4 : 1. At autopsy living fetusses had been found exclusively in those animals in which castration had taken place on the 14th day; the same finding, however, in the (castrated) controls, treated with nothing but gummi arabicum.

The evaluation of the results in the groups, castrated on the 5th day, is complicated by the fact, that the demonstration of sperm in the vaginal smear is not the *absolute* proof, that pregnancy has existed. The nidation of the ovum does not take place in the rat but on the 5th day, sothat at the time of castration the diagnosis of an eventual pregnancy cannot yet be delivered.

The "placental sign", i.e. the appearance of blood in the vaginal smear — what occasionally happens on the 8th day of pregnancy already — is another sign of pregnancy. (The absence hereof, however, does not exclude pregnancy; the latter may have come to an end before the 8th day). In 2 of the animals of each group, castrated on the 5th day this sign was present, so that at least in these cases pregnancy has certainly existed.

2. Six pregnant *rabbits* were bilaterally castrated on the 13th or 15th day. From the day before castration on, the animals received twice daily aethinyl-testosterone orally or subcut. during one week. A 7th rabbit, (also

pregnant and castrated) received 3.125 mgm *progesterone* daily. On the base of experiments reported in literature (COURRIER c.s. (12), ALLEN c.s. (13), PINCUS c.s. (14)) we expected this quantity to be amply sufficient to maintain the pregnancy of this animal. This appeared to be true: at autopsy, performed on the 7th day after castration, in both horns totally 5 living embryos were found, no dead ones, no placental remainders.

With aeth.-test., in the quantities as applied by us, we never succeeded in keeping an embryo alive. Neither the 4-fold of the active dose of progesterone, viz. daily 12.5 mgm aeth.-test. subcutaneously administered (2 rabbits), nor the 10-fold, viz. daily 31 mgm aeth.-test., orally administered (2 rabbits) proved to be able to procure the pregnancy of the castrated rabbit.

Even an increase of the dosage unto as much as 30 mgm subcut. (1 rabbit) and 80 mgm orally (1 rabbit) failed to yield any result, except placental remainders and, sometimes recognizable, fetusses.

These findings are not in disagreement with those of COURRIER's and JOST's. In their experiments 3 rabbits, castrated on the 4th day of pregnancy, received 5—10 mgm aeth.-test. orally daily; 2 other animals 20 mgm aeth.-test. daily by way of subcut. injection. Both dosages proved to be insufficient to maintain pregnancy.

A 2nd group of 4 rabbits received 40 mgm aeth.-test. subcut. daily and besides 10 mgm orally. Two of them were castrated on the 4th day and killed on the 10th or 12th. 4, resp. 8 nidated ova were found. The 2 other animals were castrated on the 12th day. One animal was killed 10 days later. Result: 1 living embryo and 5 on the way of resorption. The remaining animal was killed on the 17th day already: 9 normal fetusses were found. These experiments show that 40 mgm aeth.-test. subcut. and 10 mgm aeth.-test. orally, both daily administered, are able to make the nidation of the ovum possible in the castrate rabbit.

In one animal the authors could keep the fetus alive with this dosage during 5 days.

Our highest dosages were lower than the one with which COURRIER succeeded; the latter may probably be seen as a limit quantity.

This implies, that the relation between progesterone and aeth.-test. as found with the CLAUBERG test (1 : 4 : 8—10) does *not* pertain to the capacity to maintain pregnancy. COURRIER, when using aeth.-test. had to give the 15-fold of the active progesterone dose subcutaneously in order to keep the rabbit fetus alive. No details exist, concerning the required "oral" quantity.

e. Counteraction of oestrone in male mice: suppression of paradoxical effect.

It appeared from experiments by DE JONGH c.s. (15), that among the changes in the accessory reproductive organs of the spayed, adult mouse brought about under the influence of oestrone, one is specially counteracted by progesterone, namely the strong development of muco-reticular connective tissue around the ampulla ducti deferentis. With the aid of the qualitative and quantitative dates, resulting from these experiments, we investigated the influence of aeth.-test., orally administered, upon the

aforementioned paradoxical oestrone effect. Spayed adult male mice were injected with 1 γ oestrone, twice daily, for 3 weeks. One-half of the animals received besides daily aeth.-test., emulgated in gummi arabicum.

The rank growth of connective tissue around the ampulla after the injection of 2 γ oestrone daily for 3 weeks can be counteracted with 200 γ progesterone, subcutaneously given. The quantities of Aeth.-test., used by us and administered by way of a stomach tube, were 6—10 times as high, viz. 1.2—2 mgm daily.

The daily application of the stomach tube (in preliminary experiments even twice daily!) implies a severe trauma and caused a high mortality among our mice, so that, from 3 separate experiments, only 9 animals remained, that had been treated with a combination of oestrone and aeth.-test. and that could be compared with an equal number, treated with oestrone only. From these experiments it appeared, that the growth of the periampullar connective tissue could be slightly inhibited with 1.2 mgm aeth.-test. *per os* daily and distinctly with 2 mgm.

In view of this counteraction, the relation of the effective doses progesterone — aeth.-test. *per os* corresponds rather well with the one, found with the CLAUBERG-test.

f. Suppression of the oestrone-oestrus in female mice.

DE FREMERY, KOBER and TAUSK (16) reported, that in castrate, adult mice the oestrus, produced with 7 int. U oestrone (min. dosage for *oral* oestrus effect), was suppressed if 3 rabb. U oestronefree, non crystalline corpus luteum hormone ("progestine") was simultaneously injected (1 U: insufficient; 2 U: not experimented with).

In similar experiments we now investigated the influence of aeth.-test., orally given, on the oestrus, produced with a limit dose of oestrone, subcut. injected.

Castrate, female mice received 0.1 γ or 0.12 γ oestrone, divided over 3 injections (in 0.1 cc oil). One half of the animals received besides also in 3 injections 12 or 20 mgm aeth.-test. *per os* (stomach-tube). Vaginal smear every day, 3rd and 4th day twice daily. After a week the experiment was repeated, with interchange of the groups, in view of the given substances.

With 12 mgm aeth.-test. neither the oestrus effect of 0.1 γ nor that of 0.12 γ oestrone could be counteracted. In 3 experiments with groups of 5 animals, 8 had reached a positive oestrus index ("*e, f. g.*") at the moment of their maximal reaction [1]), under the influence of 0.1 γ oestrone, while the combination with 12 mgm aeth.-test. *per os* yielded 9 positive signs. In the experiment in which 0.12 γ oestrone had been used, the results even were resp. 4 and 11! The significance of these findings will be dealt with more closely in our next paper on the (oestrogenic) activity of aeth.-test.

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[1]) Details about this index: S. E. DE JONGH, E. LAQUEUR, P. DE FREMERY, Biochem. Zschr. **250**, 1932.

With 20 mgm aeth.-test. orally a cross-test was done (2 series, each of 4 animals); with this quantity it proved to be possible to suppress the oestrus effect of 0.12 γ oestrone. The administration of solely 0.12 γ oestrone yielded 8 positive signs and only 2, when combined with 20 mgm aeth.-test *per os*. So 20 mgm aeth.-test. *per os* corresponds with 3 rabb. U progestine, (vid. the above research of DE FREMERY c.s.), as far as the inhibition of the artificial oestrus is concerned. When expressed in rabbit U (CLAUBERG test) aeth.-test. corresponds in our experiments with ca 2—3 R. U, so that a satisfying accordance has been found. However, besides a progesterone-like activity, aeth.-test. has testosterone-like properties and it is possible to counteract the oestrogenic effect of oestrone with testosterone also. For the discussion of the possibility, that the inhibition of the oestrone-oestrus with aeth.-test., rests upon a testosterone-like activity of aeth.-test., the reader is referred to our paper, dealing with this mechanism.

Summary.

The possibility of progesterone-like properties of aethinyl-testosterone (anhydro-oxyprogesterone, pregneninolone), subcutaneously or orally administered, has been studied. Mainly the following results were obtained:

1. In the CLAUBERG test (pregravid changes in the rabbit uterus) the effect of aeth.-test., subcutan. given, is ca $\frac{1}{4}$ that of progesterone and ca $\frac{1}{8}$—$\frac{1}{10}$ after oral administration.

2. It appeared to be impossible to provoke deciduomata in the immature rat and to maintain pregnancy in the rat and the rabbit after castration with aeth.-test. subcut. and aeth.-test. *per os*, resp. in dosages of 4 and 8—10 times the quantity, sufficient for progesterone.

3. Dates from literature (COURRIER) as well as our own experiments lead to the assumption that for the maintenance of pregnancy in the rabbit dosages of aeth.-test. are required, 15 times (subcut.) and 30—40 times (*per os*) the quantity sufficient for progesterone.

4. Aeth.-test., subcutaneously or orally administered, is capable of lowering the *sensitivity for oxytocine* of the rabbit uterus in the same quantitative relation, when compared with progesterone, as has been found with the CLAUBERG test.

5. Aeth.-test. *per os* is capable of inhibiting the effect of oestrone in castrated, male mice (paradoxical effect round ampulla ducti deferentis) and in castrated female mice (oestrus) in the same quantitative relation, when compared with progesterone, as has been found with the CLAUBERG test.

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