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

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II. *Not Progesterone-like properties (oestrone-like, testosterone-like).*

In a previous paper (1) some progesterone-like activities of aeth.-test. were described. We now wish to deal with the other effects, observed with aeth.-test., grouped as follows:

1. oestrone-like,
2. testosterone-like,
3. oestrone and/or testosterone-like?

1. *Oestrone-like activities of aeth.-test.*

a. An indication for this sort of activity we found in already published experiments, concerning the suppression of the oestrone-oestrus in castrated *mice*: with a quantity of aeth.-test., not (yet) causing an inhibition, an earlier appearance of the *oestrus* was obtained. (Table I).

TABLE I.

Treatment	Number of animals in wich oestrus appeared	
	Within 24 hours after last dosage	More than 24 hours after last dosage
0.1 γ oestrone	2	6
0.1 γ oestrone + 12 mgm aeth.-test. *per os*	9	—
0.12 γ oestrone	3	5
0.12 γ oestrone + 12 mgm aeth.-test. *per os*	10	1

This table further shows, that with the combined treatment a larger number of animals reached oestrus than with oestrone alone. The acceleration of the oestrus-effect also occurred in the majority of the animals, that escaped inhibition after a higher dosage aeth.-test. For these reasons it was investigated, whether aeth.-test. alone is capable of producing oestrus in castrated female mice: 10 or more of a series of 20 animals received 12—20 mgm aeth.-test. orally or 6—10 mgm aeth.-test. subcutaneously.

*) For literature and further details vid. anew Dissertation Leiden 1940.

The rest was used as controls. In the following weeks the groups were interchanged or a pause was shoven in between treatments. We finished with 40 mgm orally or 30 mgm subcut., in the course of 5 days.

In these experiments we sometimes met with a positive oestrus sign, what has to be considered as a proof of the oestrogenic activity of aeth.-test., since oestrus fails to appear in controls, not treated with this substance. The oestrus developed early and was of short duration: obviously the progesterone-like effect predominates very soon. Besides, the same animal could not be brought to oestrus even after weeks. The histological examination yielded the explanation: aeth.-test. had produced mucification as well as cornification in the vaginal wall (sometimes even mucous cells upon a layer of cornification!) The mucification lasted more than a week.

In castrate *rats* too we could show the oestrogenic effect of aeth.-test.: 200 γ aeth.-test. subcut. or 400 γ *per os,* both daily given for 20 days to 4—5 w. old animals gave rise (besides an initial oestrus) to the return of the oestrus on the 12th—13th day in the second group.

Oestrus was also observed in pregnant rats, castrated on the 5th or 10th day and treated with daily 5 mgm aeth.-test. subcut. or 10 mgm orally. The placenta not yet being of importance at this stage of pregnancy and the only other possibility for the production of oestrogenic substances being removed (Mc KEOWN, c.s. (2)) we considered this to be a new proof of the oestrogenic power of aeth.-test.

b. The opening of the introitus vaginae, combined with oestrus is another mainly oestrogenic effect of aeth.-test., observed by us in 8 immature rats (16—19 days old), treated with 400 γ aeth.-test. subcut., twice a day for 3 days. Though the influence of the testosterone-like component cannot be wholly denied (testosterone too is capable of producing this phenomenon), the oestrogenic effect must have played the main role here, since an equal dose of testosterone caused a disclosure, appearing *later* and *without* oestrus. Progesterone, that never produces oestrus, also fails to give rise to vaginal opening, even to a later occurring one. 800—1000 γ aeth.-test. *per os,* twice daily resulted in vaginal opening without oestrus.

c. The *changes in the uteri* of the *rats* in the above investigations also pointed to an oestrone-like and partially to a progesterone-like influence of aeth.-test.

The uterus weights in the groups "aeth.-test. subcut." were distinctly higher than those in all other groups. The enlargement of the uteri with aeth.-test. *per os* was of the same range as that with testosterone.

Histologically the "aeth.-test. subcut." uteri showed a loose, vacuolated stroma with blown-up nuclei and an ephithelium of high, cylindrical cells with clear basal nuclei and a high protoplasm seam. Neither testosterone, nor progesterone, nor aeth.-test. *per os* could call forth this picture. It was only found in rats, treated with 400 γ progesterone + 0,25 γ oestrone twice

daily for 3 days or 0,25 γ oestrone 2 dd during 6 days, combined with 2 dd 400 γ progesterone the last 3 days.

d. The oestrogenic effect of aeth.-test. on the *uterus* was also seen in immature *rabbits*: 40 mgm aeth.-test. orally, divided over 5 days caused a growth of the organ, somewhat inferior to that, obtained with 10 γ oestrone subc. and somewhat superior to that with 20 mgm testosterone proprionate subc. 20 mgm aeth.-test. subcut. caused a much smaller growth; after 5 mgm progesterone (subcut.) or a combination of 8 γ oestrone and 5 mgm progesterone (subcut.) hardly any growth occurred. Aeth.-test. orally given produced, histologically followed, the same pregravid alterations (less than corresponding with the sign +) as the combination of oestrone and progesterone did.

Moreover, sensibilization of the uterus to 1 mgm progesterone proved to be possible with a precursory gift of 40 mgm aeth.-test. *per os*, just like with oestrone in the CLAUBERG test. This also could be obtained with the combination of oestrone and progesterone given simultaneously.

2. *Testosterone-like effects.*

a. Influence upon seminal vesicles and prostatic gland of the castrated, whether or not hypophysectomized rat. Castrated, 4—5 weeks old rats received 0,5 mgm aeth.-test. daily for 8 days in one or two gifts a day. At autopsy on the 9th day seminal vesicles + coagulation gland and the ventral lobe of the prostata proved to have grown distinctly more than those of the controls.

Though quantitatively much less impressive than after treatment with 200 γ testosterone daily, in qualitative sense the development after aeth.-test. was just the same ("activity" in the histological picture).

Oral administration of twice a subcut. active dosage gave no result. The fourfold caused a slight increase in weight, but no histological changes. (Table II).

TABLE II.

Daily dosage aeth.-test.	Sem. ves. and coag. gl.	Ventr. lobe prost.
2 dd 0.25 mgm subcut.	43.8 mgm (11.3 mgm)	31.6 mgm (6.5 mgm)
1 dd 0.5 mgm „	23.6 mgm (9.5 mgm)	17.8 mgm (9.8 mgm)
2 dd 0.5 mgm *per os*	9.6 mgm (9.4 mgm)	9.8 mgm (8.8 mgm)
1 dd 1 mgm „	8.8 mgm (9.5 mgm)	8.2 mgm (9.8 mgm)
1 dd 2 mgm „	13.3 mgm (9.5 mgm)	13.8 mgm (9.8 mgm)

Av. wt. semin. ves. + coag. gl. and ventr. lobe of the prost., as obtained in 2 experiments. Corresp. control wts. in parenthesis.

These experiments showed — in agreement with the findings of EMMENS' c.s. (3), pertaining to the influence of aeth.-test. on the cock's comb — that aeth.-test. orally given, doesn't unfold but a weak "masculine" activity in the male rat. From the obtained increase of the weight of the seminal vesicles it follows, that this "masculine" activity bears a really testosterone-like character and not a progesterone-like one, as might be expected because of the findings of GREENE c.s. (4). The above increase can be obtained with progesterone in massive dosages only (reaching, when expressed in rabbit U., far beyond the quantities of aeth.-test., used by us. (GREENE c.s.). In this respect progesterone corresponds with "male" hormone (type androsterone) from the urine, aeth.-test. with "male" hormone (type testosterone) from the testicles.

That the "masculine" activity of aeth.-test. belongs to the testosterone-type, also appeared from an experiment, in which 0.25 mgm aeth.-test. twice daily subcutaneously was administered for 8 days to hypophysectomized rats, 9—10 weeks of age and castrated 4 weeks before. On the 9th day the weights of seminal vesicles + coagulation gland and of the prostate were resp. av. 25,6 mgm and 9,3 mgm; of the controls resp. 8 and 4,6 mgm. FREUD and LAQUEUR (5) showed that such an increase in weight of the seminal vesicles only occurs in hypophysectomized, castrate rats, when the testosterone type is used and not with the androsterone type (even not if combined with oestrone).

The stimulation of seminal vesicles and prostata occurred, as a by-phenomenon, in one of our experiments, in which we daily administered during 20 days 400 γ aeth.-test. *per os*, 200 γ aeth.-test. subcut., 200 γ progesterone or 200 γ testosterone. Testosterone gave rise to a strong growth of the above organs, aeth.-test. subcut. to a lesser degree, but still distinct enlargement; the remaining substances had hardly any or no effect.

b. Inhibition of the paradoxical effect in the castrated mouse: The technics of the experiment have been dealt with in our previous paper; it was shown that aeth.-test. can inhibit the paradoxical alterations, that are specially suspectible for the influence of progesterone. However, also the changes, for which testosterone has a certain preference (epithelium of the coagulation gland and of the efferent ducts of seminal vesicles and coagulation gland) were reduced!

The used quantity of aeth.-test. was 1.2—2 mgm *per os* daily. The effect of the quantity of oestrone, used by us, can be inhibited with 6 γ testosterone daily, as has been shown in other experiments in our Laboratory (DE JONGH c.s. (6)). Basing hereupon, the "masculine" activity of aeth.-test. is only ca 1/200—1/300 of that of testosterone. A further sign of this "masculine" property has been found in the "male" aspect of the coagulation gland of the animals, that had received 2 mgm aeth.-test. *per os* daily.

c. Inhibition of the oestrone-oestrus in mice. These experiments too have been described already in the previous communication: aeth.-test., orally given inhibits the oestrone-oestrus and the relation with progesterone concerning the active dosages is rather well the same as is found with the CLAUBERG test. VAN DER WOERD (7), however, showed in experiments, not wholly comparable with ours, that 150 γ testosterone counteracts the oestrus effect of 0,1 γ oestrone; DE FREMERY c.s. (8) suppressed a limit dosage of oestrone with 3 rabbit U. progesterone. When comparing these dosages, one conclusevely finds the relation index (pro mgm), also fixed by ROBSON (9): testosterone inhibition-progesterone inhibition = 15 : 1.

In view of the inhibition of the paradoxical oestrone effect in male mice the activity of aeth.-test. *per os* proved to be only ca 1/200—1/300 of that of testosterone. Hence it may be expected that the oestrone-oestrus can be suppressed with 200—300 × 150 γ = 30—40 mgm aeth.-test. In our experiments 20 mgm aeth.-test. sufficed. The rough calculation basis taken into account, the inhibition of the oestrone-oestrus with aeth.-test. orally given, *might* have been due to a testosterone-like effect.

d. Influence on the rat penis and clitoris: In the above experiments on the influence of aeth.-test. on the vaginal opening in the immature rat aeth.-test. subcut. proved to be capable of causing a *clitoris growth,* stronger (!) than that with an equal dose of testosterone. Twice this quantity of aeth.-test., orally given, caused a slight increase in weight, an equal quantity of progesterone had no effect. (Table III).

TABLE III.

Daily dosage	1st experiment	2nd experiment
800 γ aeth.-test. subcut.	15.5 mgm	9.6 mgm
1600—2000 γ aeth.-test. *per os*	7 mgm	—
800 γ testosterone	8.3 mgm	5.6 mgm
800 γ progesterone	3.2 mgm	—
control	3 mgm	4.5 mgm

Average weight of the clitoris.

In experiments of 20 days duration with 4—5 weeks old, castrated rats 200 γ testosterone daily caused a larger gain in weight than 200 γ aeth.-test. subcut. did; 400 γ aeth.-test. orally caused a slight, 200 γ progesterone no growth.

The growth, in these experiments induced with aeth.-test. subcut. appeared together with a cartilagenous alteration of the clitoris of the same character as is seen in consequence of a treatment with testosterone. In long lasting experiments aeth.-test. *per os* as well as progesterone some-

times caused slight changes in the same direction. In 3 experiments progesterone only did so, when combined with oestrone.

Aeth.-test. subcut. also induced in similar experiments, in the shorter as well as in the long lasting ones, penile growth in 4—5 weeks old, castrated rats, (Table IV), which proved to be preserved in the above mentioned hypophysectomized, castrated rats.

TABLE IV.

Daily Dosage mgm		Penile wt. (mgm)
8 days	0.5 aeth.-test. subc.	48.4
	1 aeth.-test. *per os*	33
	2 aeth.-test. *per os*	34.4
	control	30.5
20 days	0.2 aeth.-test. subc.	24.7
	0.4 aeth.-test. *per os*	27.3
	0.2 testost. subc.	90.3
	0.2 progest. subc.	25
	control	24.7

Av. weight of the rat penis.

The cartilagenous transformation as found in the clitoris, was also observed in the glans penis of those animals, that had received aeth.-test. subcutaneously. To a lesser degree this was also the case after 2 mgm aeth.-test. orally during 8 days and after 400 γ aeth.-test. orally during 20 days; the changes were highly developed after the administration of 200 γ testosterone.

In the os priapi we observed modifications as described by DE JONGH c.s. (10) for testosterone. The whole penile bone was thicker than in the controls, albeit that no distinct separation in 2 layers could be shown; there was an active cartilagenous mantle of the marrow cavity, in which markedly developed trabeculated tissue was present. These changes were obtained much more distinctly with aeth.-test. subcut., than with the double quantity, orally administered.

In the penile bones of hypophysectomized, castrated rats too, changes as described by DE JONGH c.s. for testosterone-proprionate were observed.

3. *Oestrone-like and/or testosterone-like activity.*

In this group a series of changes are reported, that did not fit in the foregoing groups.

a. Influence on the intact pregnancy in the rat. With oestrogens pregnancy can be disturbed in the rat during the first half. Concerning a disturbance with testosterone (propr.) valuable details are only given in literature for the second half of pregnancy (HAIN (11), GREENE (12), etc.).

We investigated the influence of 2 dd 0,625 mgm progesterone, 2 dd 0,625 mgm aeth.-test. subcut. and 2 dd 50 mgm aeth.-test., orally administered from the 4th until and inclusive the 11th day of pregnancy to groups of 4 rats. Laparatomy on the 12th and 24th day after the detection of sperm in the vagina.

Progesterone proved to induce a (slight) lengthening of pregnancy; aeth.-test. subcut. had disturbed pregnancy in 3 and aeth.-test. *per os* in 2 cases (In the remaining cases of aeth.-test *per os* the term of delivery had been postponed!) Testosterone disturbed pregnancy in all cases.

b. Influence on the preputial glands in the rat. VAN DER WOERD (13) reported that oestrone as well as testosterone make the preputial gland grow in male and female rats all alike; with progesterone this occurs to a much lesser degree. In our experiments on male and female rats (during 3, 8 or 20 days) these glands have been weighed almost regularly. Without exception we found a slight increase in weight after aeth.-test.

c. Influence on the pituitary changes after castration. Testosterone and oestrone are capable of preventing the appearance of "castration cells" in the hypophysis, not progesterone (SCHOELLER, DOHRN and HOHLWEG (14)). The female pituitary is more sensible than the male one is. In experiments, in which 4—5 weeks old, castrated rats had been treated with testosterone, progesterone, aeth.-test. subcut. or aeth.-test. *per os*, the hypophysis was controlled at autopsy. In the following table the presence of castration cells is marked with +, absence with —, in case of only slight development ±. (Table V).

TABLE V.

Daily dosage	Castr. cells in ♂ rats	Castr. cells in ♀ rats
200 γ aeth.-test. subc.	±, +, ±	±, +, ±
400 γ aeth.-test. *per os*	±, +, +	—, —, ±
200 γ testosterone	—, —, —	—, —, —
200 γ progesterone	+, +, +	+, +, +
control	+, +, +	+, +, +

In the female (more sensitive!) rats aeth.-test. *per os* prevented the appearance of castration cells. Our material does not allow an explanation of the failure of "aeth.-test. subcut.". In our pregnant rats, however,

castrated on the 5th day 5 mgm aeth.-test. subcut. and 10 mgm aeth.-test. *per os*, both daily given, did *not* fail to suppress the development of castration cells!

d. Inhibition of lactation in mice. Oestrone and testosterone are capable of inhibiting lactation (DE JONGH c.s. (15), ROBSON (16)). We took the body weight of the young as an index, but could not show that way any inhibition with 400 γ aeth.-test. subcut., 800 γ aeth.-test. *per os* or 400 γ progesterone subcut., daily given from the 1st until and inclusive the 21st day after parturition. With testosterone, conversely, we could induce a demonstrable inhibition (400 γ daily).

Discussion.

In our previous communication a series of experiments were described, with which a number of progesterone-like properties of aethinyl-testosterone were clearly shown, in good agreement with earlier publications on the subject. The present paper deals with a number of different properties of aeth.-test., characterized by us as "oestrone-like" and "testosterone-like", thus treading in the footsteps of EMMENS and PARKES.

The testosterone-like properties, in view of the structural formula of aeth.-test., need not raise our astonishment; the oestrone-like activity, however, was more or less startling news. The spreading of both effects over the various effector organs is irregular, as results from the communicated data. Thus it cannot be said in anticipation of the relative experiment, where and to which degree the oestrone-like resp. testosterone-like powers will be expressed. Neither it will be possible to draw from experiments with animals of one species conclusions for the other and in how far man is susceptible for these influences remains questionable.

In clinical literature, though scarce untofar, no facts are communicated, from which it could be deducted, that the aforementioned non-progesterone-like effects of aeth.-test. also are important for man and eventually might contain an argument against the application of aeth.-test. instead of progesterone in medical practice.

We are aware, that with the progress of clinical experience this state of affairs may change. In every case, however, the combined occurrence of three types of activity in one single sterole derivative seems us to be of sufficient scientific interest, as to justify the publication of the results of our experiments.

Summary.

Besides progesterone-like activity aethinyl-testosterone has some other properties.

A. Oestrone-like effects.

1. Quantities of aeth.-test., inadequate to inhibit oestrus, advance and corroborate the oestrone-oestrus in spayed mice.

2. It is sometimes possible to bring about oestrus in spayed mice with the aid of aeth.-test.; in those cases cornification of the vaginal wall can be shown histologically; because of the progesterone-like activity of aeth.-test., however, this effect is soon overshadowed by mucification. In, wether or not pregnant, spayed rats too, aeth.-test. produces oestrus.

3. Aeth.-test. causes vaginal opening in the immature rat, together with oestrus. This effect may be partially "testosterone-like".

4. In the uterus of immature rats, aeth.-test. brings about alterations, similar to those, induced with oestrone + progesterone in certain combinations.

Aeth.-test. promotes the growth of the immature rabbit uterus (with traces of pregravid changes!) and sensibilizes it to subsequently given progesterone.

B. Testosterone-like effects.

1. Aeth.-test. causes growth of the sex organs (seminal vesicles, coagulation gland, prostata, penis) in the castrated, whether or not hypophysectomized, immature rat, qualitatively in the same sense as testosterone does.

2. The relation progesterone : aeth.-test. subcut. : aeth.-test. orally = 1 : 4 : 8—10. as found in the CLAUBERG test for the rabbit does not hold for the testosterone-like effects in the rat. This effect is after oral administration relatively much less pronounced. The testosterone-like activity of aeth.-test. does *not* rest upon its progesterone-like properties, but is wholly independant.

3. In the immature rat aeth.-test. causes growth of the clitoris, exceeding that, following the administration of the same quantity of testosterone.

4. Aeth.-test. inhibits paradoxical effects of oestrone in the male castrated mouse, that are electively sensitive to testosterone.

The inhibition of the oestrone-oestrus in mice may also be due to testosterone-like activity of aeth.-test.

C. Oestrone- or testosterone-like effects.

1. Aeth.-test. disturbs the intact pregnancy in the rat.

2. Aeth.-test. causes growth of the preputial glands in male and female rats.

3. Aeth.-test. prevents the development of "castration cells" in the pituitary of the spayed rat.

4. 400 γ aeth.-test. daily subcutaneously or 800 γ aeth.-test. daily orally does not inhibit lactation in nursing mice.

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