CXCV. CONDITIONS OF HYPERTROPHY OF THE SEMINAL VESICLES IN RATS.

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PART I.

VOSS AND LOEWE [1931] and Moore and Gallagher [1930] have described certain restoration phenomena in secondary sex organs of castrated mice and rats respectively, by treatment with male hormone, the former authors being the first to use changes in the epithelium of the seminal vesicles as a rapid cytological test for the action of the hormone. Complete restoration was claimed by Moore and Gallagher [1930] to result from administration of 5 capon units (as defined by Gallagher and Koch [1930]) daily for 21 days, this daily dose being equivalent to 7.5 capon units as defined by Freud *et al.* [1932].

As will appear from the work to be described below, the male hormone is only one of the hormones which act on the seminal vesicles, of which it causes the epithelium to hypertrophy; the complete restoration claimed by the above mentioned authors is impossible with male hormone alone and was probably obtained by the use of preparations contaminated with oestrin or its homologues, these substances alone being stimulants of smooth muscular hypertrophy.

In this laboratory particular attention has been paid to parallel experiments on capons and on rats. The reaction of capons to preparations of differing purity and obtained from various sources proved to be reliable in our hands, contrary to statements of Korenchevsky *et al.* [1933], who reject the capon method as unreliable, and advocate testing on rats. To their statement the same criticism applies as in the case of Moore and Gallagher [1932].

In capons general conditions such as sunlight, health of the animals and, according to de Fremery [1931], thyroid function during various seasons as well as the male hormone influence comb growth. It must be especially pointed out in this connection that ketohydroxyoestrin (menformon) in crystalline form up to 1000 M.U. a day has no influence whatsoever upon comb growth. The seminal vesicles on the other hand are distinctly influenced by menformon or its homologues and as will be shown in Part II, these substances may be important in facilitating the action of male hormone at comparatively low doses of the latter.

On account of the irregular response of the seminal vesicles to various preparations, it was impossible to establish a constant ratio of minimum active quantities of male hormone between rats and capons. Continued fractionation of extracts led to their gradual loss of potency with reference to seminal vesicles without corresponding loss of potency in capons. The preliminary assumption of a separate comb growth and seminal vesicle hormone was soon replaced by another conception, which will be presented below.

Choice of material.

Seminal vesicles are easy to prepare and they respond rapidly to castration or treatment. They may serve as indicators of the hormonal state of the genital system, although there are other organs, such as the preputial glands of rats, the reaction of which is not conditioned by the presence of some other active substance though it is less specific than that of seminal vesicles. With these organs we propose to deal later.

The weight of the seminal vesicles of adult rats ranges between 120 and 800 mg. varying with (1) secretory state, (2) size of testicle, (3) structure of testicle, (4) size of hypophysis, thyroid and adrenals, (5) general condition of the animal. After castration of adult rats the weight of the seminal vesicles varies from 90 to 350 mg. The atrophy does not reach a definite basic point even after months. There is a marked difference between rats castrated during infancy or later; in the former the seminal vesicles cease to grow at once after removal of the testicles. Whilst a complete restitution of the comb and other male characters was obtained in capons by continued treatment with male hormone [Freud *et al.*, 1932] it is difficult for male hormone to act on completely atrophic seminal vesicles, just as progesterin [Corner and Allen, 1929] is potent only after preparation of the muscular tissue of the uterus of infantile rabbits by oestrin. The seminal vesicles of rats castrated during infancy may be taken therefore as indicators, not of male hormone only, but of complete testicular activity. This idea is supported by experiments on senile and on non-castrated infantile male rats with gonadotropic substances, which, stimulating the whole of the testicle, lead to an almost complete (precocious) development of the seminal vesicles [de Jongh, 1930].

On these grounds we decided to use infantile rats as test material and simplified the method by beginning their treatment almost immediately after castration, instead of awaiting maturity. In these animals we aimed at forcing a precocious development of secondary sex characters. Under these circumstances the weight of the seminal vesicles is the first indicator of the effect of a certain treatment, the histological picture being used for the analysis of their growth.

Technique.

25–45 g. (3–5 weeks old) rats are castrated by the transscrotal route. There is no bleeding; one stitch closes the wound. This operation has the object of preventing spontaneous genital development during the experiment. Three days later a course of 8 injections is started, litter-mates as far as possible being equally distributed between experimental and control groups. Twice a day 0.1 cc. liquid is administered subcutaneously. On the day following the last injection the animals are killed with chloroform. Both seminal vesicles are cut out in one semilunar piece and weighed on a torsion balance immediately after removal. A narrow bridge of prostate tissue connects the two lobes. Formaldehyde is used for fixing and haematoxylin-eosin for staining.

Histology of seminal vesicles.

Seminal vesicles have a peritoneal covering, connective tissue stroma with smooth muscle tissue imbedded therein, a small zone of subepithelial stroma and a single layer of cylindrical epithelial cells lining a sinuous cavity. In moderate hypertrophy the sinuosities are macroscopically visible.

Hypertrophy. Muscle cells large, with bright nuclei. Frequent mitoses. Epithelium high cylindrical, with basal nuclei reaching to 1/3 or 2/3 of the total height of the cells and leaving

a protoplasmic margin free towards the cavity. Mitotic cell divisions characterise the beginning of hypertrophy, they diminish later on. The cavity contains basophilic, homogeneous secretion.

Atrophy. Small muscle cells with dark nuclei, low epithelium of cubical cell form, without protoplasmic margin, the nuclei filling the cell bodies almost completely. No mitoses, only sporadic pycnosis of nuclei, imitating cell division.

Muscular hypertrophy does occur without epithelial development, while epithelial hypertrophy without a certain muscular development was never observed.

Apparent paradoxical effect of menformon.

In a previous paper [Freud *et al.*, 1933] muscular hypertrophy of seminal vesicles under the influence of menformon (keto-hydroxyoestrin) was described under this heading. Male and female hormones are produced in both sexes in approximately equal quantities, as judged by their excretion in the urine of males and non-pregnant females. The two substances are distinctly different pharmacologically, they act on different tissues. The effect of menformon in males is only apparently paradoxical.

Weight of seminal vesicles.

In Table I 32 untreated castrated male animals are classified according to body weight and weight of the seminal vesicles.

Table I.

Body weight g.	Weight of seminal vesicles (mg.)							Total
	3-4	4-5	5-6	6-7	7-8	8-9	9-10	
21-25	—	2 (Feb.)	1 (Feb.)	—	—	—	—	3
26-30	1 (May)	1 (July)	—	—	—	1 (July)	—	3
31-35	—	—	—	1 (July)	2 (July)	—	—	3
36-40	1 (July)	1 (May)	1 (Jan.) 1 (May)	1 (Mar.) 2 (May)	2 (Jan.) 2 (Feb.)	— 1 (May)	—	13
41-45	—	—	—	1 (Feb.) 1 (July)	1 (Feb.) 1 (May)	—	—	4
46-50	—	—	—	—	1 (Feb.)	1 (Mar.)	—	3
51-55	—	1 (Feb.)	—	—	1 (Mar.)	1 (July)	1 (May)	3
Total	2	5	3	6	10	4	2	32

From this table we may conclude that seminal vesicles weighing more than 10 mg. scarcely ever occur in animals of less than 55 g. Wallen-Lawrence and van Dyke [1931] state that infantile non-castrated rats below 40 g. body weight have seminal vesicles of an average weight of 9.24 mg. Our material with reference to non-castrated rats confirms this statement. Geographical and racial differences do not seem to have any noticeable influence. The seminal vesicles of castrated and of intact infantile males between 25 and 45 g. body weight practically do not differ. Histologically no large difference exists either, with the possible exception that nuclear pycnosis occurs in castrates only.

How does male hormone act?

Assuming that the histological state of the epithelium of the seminal vesicles decides whether a male hormone preparation is potent or not, the question of the relation between weight and histology arises. The degree of epithelial response may be classified according to two criteria: height of epithelium and number of mitoses. In this paper however the response is considered as a whole without further differentiation. A response is positive whenever the protoplasmic margin exceeds one-third of the cell height or when at least one mitosis is discovered per visual field at a moderate magnification.

The photomicrographs represent seminal vesicles of infantile castrated male rats. 1, 2 and 3 all at the same low magnification.

Fig. 1. Without treatment; atrophic smooth muscle and epithelium.

Fig. 2. Treatment with menformon; hypertrophic smooth muscle, atrophic epithelium.

Fig. 3. Treatment with male hormone combined with menformon; totally hypertrophic seminal vesicle with ramified lumen and secretion.

Fig. 4. The same preparation as Fig. 3 at higher magnification, showing details of hypertrophic epithelium (note mitoses).

In Table II 940 animals are classified according to weight of seminal vesicles and positive or negative response of the epithelium. From this we see that epithelial responses become positive between 16 and 19 mg., while above 20 mg. with one exception, no negative response occurs. Positive responses below 14 mg. are exceptional. From this table it appears reasonable to assume 19 mg. as the *positivity limit*.

Table II.

Weight of seminal vesicles in mg.	Epithelial response		Percentage		Number of animals
	Positive	Negative	Positive	Negative	
4-5	0	16	0	100	16
6-7	0	36	0	100	36
8-9	1	68	0.15	99.85	69
10-11	3	109	2.7	97.3	112
12-13	9	125	6.7	93.3	134
14-15	26	121	17	83	147
16	13	56			
17	21 34	38 94	27	73	128
18	28	17			
19	32 60	3 20	75	25	80
20-21	69	0	100	0	69
22-23	37	1	98	2	38
24-25	27	0	100	0	27
26-27	17	0	100	0	17
28-29	14	0	100	0	14
30-31	7	0	100	0	7
32-33	4	0	100	0	4
34-35	4	0	100	0	4
36-37	4	0	100	0	4
38-39	3	0	100	0	3
40 and more	31	0	100	0	31
Total	350	590	—	—	940

Sixty-four animals are not classified in Table II, because the treatment of these marks them as a special group. Their epithelial response was consistently negative, although the weights of their seminal vesicles were between 14 and 30 mg. They were treated with one of the following kinds of preparations: (1) unpurified or crystalline oestrin (menformon); (2) crystalline menformon *plus* subthreshold doses of male hormone; (3) oestrin fractions of male urine after separation from male hormone.

Table III shows the distribution of the cases according to the weight of the seminal vesicles.

Table III.

Weight of the seminal vesicles in mg.	Number of (negative) epithelial responses
14-15	1
16-17	4
18-19	28
20-21	13
22-23	9
24-25	6
26-27	2
28-29	0
30	1
Total	64

From Tables II and III, the following conclusion seems justified. In low doses male hormone is inert. In comparatively high doses, in seminal vesicles of above 14 mg. weight, the frequency of "positive" epithelial responses increases

rapidly until above 19 mg. practically all the reactions are positive. However, there are doses of male hormone insufficient to produce epithelial changes but just sufficient to help oestrin in producing muscular hypertrophy. Hence in seminal vesicles between 14–30 mg. histological control is advisable to detect whether the effect is merely due to oestrin and subthreshold doses of male hormone, or whether the dose of male hormone was sufficient to cause epithelial hypertrophy. Of course not all animals respond to threshold doses.

Reliability of the reaction.

Three criteria are decisive with reference to this question.

(1) *Individual response variations within one group of experimental animals.* This criterion is in favour of reliability in a large majority of the seminal vesicle reactions.

(2) *Increasing response to increasing doses.* Only those experiments are to be used for comparison which were carried out at the same time, so as to exclude group variations due to circumstances changing with time. 126 serial experiments with two or more doses and with exclusion of all those cases where all doses were subthreshold yielded the following result:

	Times	Percentage
Higher dose—increased response	87	69·05
Different doses—equal response	24	19·05
Paradoxical result, higher dose, weaker response	15	11·9

The doses varied as 1:2:4:8 and so on. A group response of otherwise comparable animals was considered different when weight differences of the seminal vesicles averaged more than 2–3 mg., or when histology gave the impression of higher intensity of response.

(3) *Reproducibility of results.* This criterion includes the time factor of response. 57 times threshold doses of the same fractions of a preparation were administered repeatedly to groups of rats during different weeks. 21 experiments were discrepant, 36 yielded identical results. Of these latter however both experiments were negative 16 times and so of doubtful value in this connection. Our last criterion is thus unsatisfied, at least as long as the exact amounts of oestrin contaminating our preparations are unknown.

Influence of body weight and growth.

In Table IV the influence of body weight upon the growth of seminal vesicles is indicated. This table is constructed as follows: 3 animals of one experiment are classified as “light,” “medium” and “heavy” in the corresponding horizontal

Table IV.

Weight of animals	Weight of seminal vesicles			
	Light	Medium	Heavy	
Light	235	95	107	Total: 1189
Medium	109	109	88	
Heavy	107	75	264	

lines of the table, in the columns according to the weight of their respective seminal vesicles. Every animal is placed by comparison with the two others of the same experiment. When two animals of a group have equal weights, they are placed in the “light” and “heavy” rows in the columns corresponding to their respective seminal vesicles.

Table IV refers to 1189 animals, treated with potent preparations, showing a marked tendency to respond better with increasing weight. By considering the diagonal of this table from left above to right below as the median line of normal reactions and taking the sum of the figures along it and along the lines parallel with it, the following frequency range results: 107, 184, 608, 183, 107. This is a normal or a Gauss frequency curve, in which the summit is along the diagonal connecting those values which show that larger animals tend to react more strongly than small ones.

Similar conclusions may be reached by considering the influence of growth of the animal during the experiment upon growth of the seminal vesicles. This is shown in Table V.

Table V.

	Weight of seminal vesicles			
Growth of animals	Light	Medium	Heavy	
Small	221	84	148	
Medium	81	81	84	
Great	143	99	220	Total: 1161
Frequency distribution along diagonals: 143, 180, 522, 168, 148.				

From these figures we see a tendency for the seminal vesicles to grow most in animals which themselves grow most.

The conditions for optimum growth of seminal vesicles are present in comparatively large and actively growing animals. By introducing body growth as essentially affecting seminal vesicle response to treatment, the existence of a number of external and internal factors is revealed in these experiments. These again account for the extraordinary variability of the reaction and for the difficulty of reproducing threshold reactions at different times. The influence of hypophyseal (growth?) factors upon this reaction will be discussed in another paper. The necessity of a large number of experiments preceding any kind of consistent conclusion is explained by these circumstances.

PART II.

This part deals with the experimental determination of the rôle of the follicular hormone (oestrin) in the development of seminal vesicles.

Varying potency of capon units of male hormone in rats.

Forty-one preparations and fractions of male hormone, extracted from testicles and from male urine, were tested on capons and on rats. In Table VI the comparative values of potency in these two kinds of animals are noted. Standardisation could not be done completely in all cases, the probable minimum active quantity is in many cases more or less than the one noted in the table, both in rats and in capons. Yet from the figures it is apparent that a more exact determination would have made the irregularity of response of rats in most cases still more marked.

A large number of preparations, obtained by the same procedures as those noted in Table VI, were tested on rats only. While the quantity of male hormone in such preparations, whether obtained from urine or from testes, when determined by the capon test, became with increasing experience almost constant,

Table VI.

Prep. from testis	g. tissue con- taining 1 capon unit	g. tissue con- taining 1 rat unit	1 capon unit equal to rat units	Prep. from urine	Litres urine con- taining 1 capon unit	Litres urine con- taining 1 rat unit	1 capon unit equal to rat units
107	100	100	1.0	120 (II)	2.0	0.4	<0.2
112	20	10	0.5	139 (I)	0.5	>0.5	>1.0
113	50	150	3.0	149 (I)	0.5	0.8	1.6
114	100	>20	>0.25	178 (I)	0.5	0.4	?1.0
131	80	20	0.2	178 (II)	5.0	>1.0	?0.2
141	50	15	0.3	294 (II)	1.0	>1.0	>1.0
143	50	20	0.4	315	2.0	2.0	1.0
147	25	40	1.6	315 (II)	2.0	>4.0	>2.0
167	<?50	50	>1.0	Z 12	<0.25	<2.0	?0.1
273	50	>50	>1.0	Z 15	2.0	0.1	0.05
Z 4	50	25	0.5	Z 16 (I)	1.0	>0.4	>0.4
Z 7	25	25	>1.0	Z 22	0.2	0.2	1.0
Z 13	25	>40	>1.6	Z 26 (I)	3.0	≈0.4	0.14
338	50	80	1.6	Z 26 (II)	3.0	>0.4	>0.14
358	50	>50	>1.0	390	2.0	>0.4	>0.2
Ho	50	80	1.6	390 (II)	2.0	>0.4	≈0.2
470	100	≈100	>1.0	390 (II)	2.0	>0.4	≈?0.2
491	50	>50	>1.0	455	0.5	>0.4	>0.8
510	50	>50	>1.0	455 (II)	0.5	0.2	>0.4
				459	0.5	>0.2	>0.4
				459 (II)	0.5	>0.2	>0.4
				514	0.5	0.8	?1.6

the potency of such preparations in rats remained very variable; hence the chemical procedure applied for the preparation of male hormone active in birds must be considered inadequate for obtaining a product, which is fully active in rats. (According to recent results, by improved methods the number of bird units found in testes is about 60 units per kg.; in urine about 30 units per litre.)

Pace-making.

In previous sections the importance of ketohydroxyoestrin for seminal vesicles has been emphasised. Besides this form of oestrogenic material, other substances have been described by Marrian [1930], Butenandt and Marrian [1931], Doisy and Thayer [1931], Girard *et al.* [1932], de Jongh *et al.* [1931] and others, which are chemically related to the original follicular hormone (menformon), but have different oestrogenic potencies. The procedure of purification of male hormone includes the elimination of these substances as well as of menformon. Quantitative data with reference to the biological potency of these substances are scanty, owing to their incompletely defined biological effects. The experience gathered in experiments such as are noted in Table VI led to systematic study of the influence of elimination of oestrogenic material and homologues from purified male hormone preparations. It was ascertained that their loss of potency was related somehow to their freedom from oestrogenic material. Preparations, which as crude extracts were potent both in rats and in capons, became inactive in rats in both fractions after separation of the male from the follicular hormone, while the fraction containing the male substance when tested in capons, proved to be almost as potent as the crude extract. This observation led us to the attempt to recover the original activity in rats by recombining the two isolated fractions, after some purification of both in the separated state. This procedure was successful. In Table VII an example is given. The fraction used was the same as referred to already in Table VI under Z 26.

Table VII.

Fraction of urine extract	Daily dose in litres of urine	mg. seminal vesicles found in rat			Histology of epithelium
		I	II	III	
I (male hormone)	0.1	9	9	—	—
	0.2	10	10	—	Negative
	0.4	12	7	9	—
II (follicular hormone)	0.1	14	11	14	—
	0.2	11	16	—	Negative
	0.4	—	19	16	—
III (I + II)	0.1	23	13	—	—
	0.2	24	20	—	Negative
	0.4	27	25	—	—

From this table we see a considerable weight increase of the seminal vesicles after recombining the two fractions of the same preparation. The epithelium of these organs was "negative," while the smooth muscular tissue of group III in all cases yielded the picture of an intensive menformon effect. From Table VI it can be seen that Z 26 was a very weak preparation as far as male hormone is concerned and compared with other extracts of urine. (In Table VI the preparations marked with II are oestrin fractions, these being almost inactive in capons.) Apparently a small amount of male hormone, insufficient to affect the epithelium, may reinforce considerably the effect of oestrin upon the smooth muscle tissue of the seminal vesicles.

In Table VIII a case of combination of male hormone extracted from testicles with a menformon fraction of urinary extract is presented. (The latter is the same as in Table VII.)

Table VIII. *Preparation 338 and Z 26 (II).*

Capon units daily of 338	Urine litres equivalent daily of Z 26 (II)	mg. seminal vesicle found in rat		
		I	II	III
0.2	—	17	9	7
0.4	—	17	14	12
0.8	—	14	17	10
0.2	0.1	20	25	21
0.4	0.2	24	25	28
0.8	0.4	30	34	40

This experiment was done in July 1931, when we still thought that seminal vesicles weighing more than 19 mg. have always a "positive" epithelium. Later a few of these organs were investigated again. It was found that the one weighing 30 mg. (see Table VIII) also had "negative" epithelium. Again male hormone of relatively low potency reinforced the effect of oestrin upon smooth muscle tissue. The final proof that the effect of male hormone was also benefited by the combination with menformon, was obtained from experiments where male hormone was combined with crystalline menformon. These experiments are presented in Table IX. In this Table, whenever histological investigation was done, the negative (—) or positive (+) epithelial response is noted.

In Table IX only 27 parallel experiments on 3–12 animals each are presented, while 11 preparations of male hormone of varying purity were tested. These preparations were combined with doses of crystalline menformon varying from 6 to 100 units a day. A large number of similar experiments done later confirm these results.

Table IX.

Preparation of male hormone	Capon units daily	Average weight seminal vesicles in mg.	Histol. of majority of cases	Additional menformon m.u. daily	Average weight seminal vesicles in mg.	Histol.
153	0.75	7.8	—	6	7.5	—
167	2.0	29	+	6	24	—
338	0.4	12	.	10	12	—
167	1.0	20	+	15	15	—
514	0.8	12	—	20	11	—
149 I	0.2	7.5	—	30	8	—
167	0.2	11	—	30	16	+
358	0.25	10	—	40	21	—
358	0.5	14	—	40	20	.
338	0.4	10	—	50	17	+
358	0.25	11	—	50	17	—
147	0.4	17	—	60	22	+
358	0.25	11	—	60	28	.
358	0.5	14	—	60	22	.
470	1.0	6	—	60	15	—
470	0.8	6	—	60	13	—
491	1.0	19	.	60	20	+
491	0.75	10	—	60	15	—
455	0.2	10	—	60	15	—
459	0.17	10	—	60	19	+
459	0.2	9	—	60	14	? +
459	0.4	7	—	60	16	+
459	0.8	9	—	60	16	+
459	1.6	17	+	60	26	+
338	0.2	11	—	100	14	.
338	0.4	12	—	100	23.5	+
338	0.8	14	.	100	24	.

Less than 40 M.U. of menformon seem to have no effect when combined with male hormone. 60 units a day regularly reinforce the seminal vesicle reaction (of another 30 similar experiments 23 showed this phenomenon). The number of positive epithelial responses also increases, though less regularly. 0.4 capon unit of male hormone without oestrin hardly ever produces seminal vesicles of more than 14 mg. in 5 days, while 1.6 units a day are a dose, which with some regularity yields appreciable growth of the seminal vesicles without the aid of oestrin. In the majority of cases 0.4 unit, reinforced by 60 units of menformon, is sufficient to produce the weight of seminal vesicles at which positive epithelial response seems to be possible. From these data we may conclude that menformon increases the effect of male hormone about 4 times. A simple addition could not explain this increased reaction, first because menformon has no effect upon the epithelium of seminal vesicles even in much larger doses than those here applied, and further because 60–200 M.U. of menformon alone tested in 24 animals yielded only exceptionally seminal vesicles of more than 11.6 mg. in 5 days.

Pace-making may thus be characterised as follows: our male hormone preparations, having a very much reduced content of menformon (as tested in castrated mice) act together with crystalline menformon upon the smooth muscle tissue of seminal vesicles. The two substances mutually reinforce each other's activity in this respect. Epithelium of seminal vesicles is influenced only by male hormone in a positive sense. This effect seems to depend on many accessory conditions. One of these is a certain dimension of the non-epithelial tissues of the seminal vesicles, seeing that according to our experience the frequency of positive epithelial responses increases rapidly in seminal vesicles of more than

19 mg. weight. The considerable number of cases in which (at least in infantile rats), in spite of comparatively large seminal vesicles, no positive epithelial response occurred proves that the size of seminal vesicles and a certain amount of male hormone are not yet always sufficient for a positive epithelial response, therefore this cannot be considered as a reliable test for male hormone as long as the rôle of other effective agents is not elucidated.

Besides menformon trihydroxyoestrin (Marrian) (crystals of Marrian) was tested in pace-maker experiments. Two preparations of male hormone (338 and 358) were used in these experiments. Their effect, when given alone, may be seen in Table IX. Table X shows the results of these experiments.

Table X.

Preparation of male hormone	Capon units daily	γ -Trihydroxy-oestrin daily	Average weight in mg. of seminal vesicles	Histology
338	0.4	1	14	—
338	0.4	3	12	—
358	0.3	4	15	—
358	0.3	5	17	.
358	0.5	5	22	.
338	0.4	5	15	+
338	0.3	5	11	—
338	0.3	6	17	.
358	0.2	10	20	+
338	0.4	10	18	+
338	0.8	10	21	+
338	0.4	20	15	+
338	0.8	20	18	+

From these data the conclusion seems justified that trihydroxyoestrin, when combined with male hormone, reinforces seminal vesicle response to about the same extent as menformon (ketohydroxyoestrin). A contamination of the preparation of trihydroxyoestrin with menformon could not possibly account for this result, because both preparations were tested on castrated female mice and the oestrogenic potency of the trihydroxy preparation was so weak that 20 γ did not correspond to more than 1.5 γ menformon, *i.e.* about 15 units. This amount of menformon is too little for pace-making. The possibility of contamination of both with a very active third substance still deserves consideration.

For trihydroxyoestrin this is a new biological effect, which if confirmed might serve as a method of testing. The fact that related compounds with comparatively low oestrogenic potency have about the same pace-maker effect as menformon, might perhaps account for comparatively strong responses of seminal vesicles to crude testicular extracts, while the testicle is known to contain very little actual oestrogenic substance. It has hitherto been impossible to determine quantitatively the non-oestrogenic compounds in various organs on account of the already mentioned lack of an adequate biological method for testing them. Trihydroxyoestrin when injected alone has, in doses of 4–10 γ daily, the same effect as similar doses of menformon, *i.e.* the seminal vesicles show a slight muscular hypertrophy, and their weight reaches in 5 days an average of 11.5 mg.

An attempt to analyse pace-making.

The following experiment was done in order to ascertain whether pace-making depends on simultaneous administration of the two hormones involved or not. 30 rats were castrated, 15 of these were treated during 6 days with 60 m.u. of menformon daily, 15 left without treatment. On the 7th day the first group of 15 was divided into three groups of 5, their treatment with

menformon was stopped, they were treated daily, as usual, with 0.2, 0.4 and 0.8 capon unit of male hormone respectively, while the group of 15 animals, which were hitherto without treatment, was also divided into three groups of 5, to which the same doses of male hormone as to the first three groups and additional 60 units of menformon daily were administered. On the 5th day of the second stage of this experiment the animals were killed. The result is shown in Table XI.

Table XI.

Capon units daily	Average weight in mg. of seminal vesicles after treatment	
	In 2 stages	Simultaneously with 2 hormones
0.2	11	15
0.4	12	17
0.8	17	19

Only one of these groups showed histologically positive epithelial response, viz. that treated simultaneously with menformon and with the highest dose of male hormone.

A treatment with menformon preceding that with male hormone does not yield the same strength of pace-maker effect as does the simultaneous treatment with both hormones.

Anomalous histological phenomena.

In 58 groups of 3 or more animals treated by mixtures of male and follicular hormones, the seminal vesicles exhibit a partial detachment of their epithelial lining from the wall of the organs. The rounded cells are conglomerated in the centre of the lumen. This result occurred only twice after exclusive treatment with male hormone extracted from testicles; it was observed 56 times in cases where male hormone was combined with crystalline or non-crystalline oestrin and with trihydroxyoestrin. By prolonging combined treatments the regularity of this occurrence is enhanced. The phenomenon hardly ever occurred where a "positive" epithelial response was obtained with male hormone. It is ascribed to an unbalanced influence of oestrin. Similar epithelial changes, not unlike tumours, were observed in rabbits' uteri after a treatment with several thousand units of follicular hormone for a few months. Lately de Jongh in our laboratory (unpublished results) observed not only epithelial degeneration, but also a metaplasia from columnar to stratified epithelium in the proximal part of the seminal vesicles of mice and rats after prolonged treatment with oestrin. This significant result will be extended and published in due course.

DISCUSSION.

An optimum growth of seminal vesicles under artificial conditions is not to be obtained by treatment with one hormone only (*e.g.* the male hormone). The effect of this substance upon smooth muscle tissue is only moderate, its main point of attack being the columnar epithelium. Oestrin and its various homologues, of which only one is mentioned in this paper (trihydroxyoestrin), act upon smooth muscle tissue, while they either do not affect columnar epithelium or induce degeneration. The structures resulting from forced treatment with these substances are somewhat reminiscent of tumours and open a possible development of tumour pathology.

The combined effect of follicular and male hormones upon seminal vesicles is a mutual reinforcement of each other's actions. For this phenomenon the

expression "pace-making" is suggested. Complete restitution of seminal vesicles in animals castrated while they were immature and treated after they became adult was not obtained even by combined treatment. This fact indicates already that compounds should be sought amongst the homologues of oestrin, which are still more potent than the ones used. We surmise that one of the homologues of oestrin might be more potent with reference to smooth muscle hypertrophy than oestrin itself and might account for the higher potency of crude extracts of male hormone than of its purified forms. It is reasonable to assume that natural development of male secondary sex organs is governed by two types (male and follicular) of hormones. It is obvious that for the measurement of the oestrin homologue active towards seminal vesicles, its oestrogenic potency gives no reliable basis. Hence we are unable to say without analysis of the effect on seminal vesicles of castrates, how much of these compounds does occur in testicles. This conception inaugurates a new line of research on "male hormones." It is quite certain that experiments, such as *e.g.* those of Moore [1932], where "complete" restitution was obtained, the preparations used must have contained besides male hormone the active principle which is highly potent in producing muscular hypertrophy. The same applies to other muscular organs, such as the vas deferens, also studied by Moore and by others.

Under these circumstances there is no question of a standardisation of "male hormone" (under this name being understood the substance acting upon the comb of capons) in these experiments. The use of rats for such a purpose though not impossible, could not be recommended before these matters are settled by careful biological analysis.

A further conclusion from these results is that antagonism of sex hormones within the secondary sex organs certainly does not exist, at least between male and female hormones. They do not inhibit each other's effects, but on the contrary, in adequate doses, they support each other (pace-maker effect). The noxious influence of an unbalanced treatment with one of these substances may be alleviated or even turned to advantage by administration in adequate doses of the other one, but in no way is this balancing based upon inhibition.

The therapeutic application of one of these substances may fail without simultaneous application of the other.

The successful application of combined male and female hormone preparations in the same proportion as mentioned above by van Cappellen [1933] in the treatment of prostate hypertrophy confirms the last statement from the side of clinical experience.

SUMMARY.

1. Simultaneous experiments on capons and on infantile male rats showed loss of activity of male hormone after purification as regards rats, while activity was retained as regards capons.

2. Oestrin promotes smooth muscle development in the secondary sex organs of mammals, hence its "paradoxical" positive growth effect with reference to seminal vesicles is only apparent. Male hormone is active in promoting epithelial hypertrophy in the same organs.

3. In hypertrophied seminal vesicles histology decides between these two types of effect.

4. Experiments on infantile rats are reliable as regards individual response in one group of animals, less so with reference to dose-action ratio, while weekly response variations are rather considerable.

5. Body weight and growth during the experiments have a marked effect upon the response to potent preparations.

6. Male and follicular hormones support each other's effects upon seminal vesicles. This is called "pace-making."

7. Trihydroxyoestrin, though its oestrogenic potency is much less than that of ketohydroxyoestrin (menformon), is an effective pace-maker in the same doses (6–10 γ) as menformon. Our knowledge about the quantities of these and homologous substances occurring in organs and tissues is scanty.

8. Abnormal histological responses of the seminal vesicles may occur after treatment of the animals with inadequate combinations of sexual hormones. The significance of these phenomena, reminiscent of tumours, remains unexplained.

9. In clinical practice, the application of these substances is advised on grounds of symptomatology and the knowledge of their pharmacological effects, rather than on so-called "hormone-balance," which does not give any idea about the mutual influence of these substances. Therapeutic application of combined male and female hormone preparations in the treatment of prostate hypertrophy has proved successful.

REFERENCES.

- Butenandt and Marrian (1931). *Z. physiol. Chem.* **200**, 4.
 Cappellen (1933). *Deutsch. med. Woch.* **19**, 726.
 Corner and Allen (1929). *Amer. J. Physiol.* **86**, 88.
 Doisy and Thayer (1931). *J. Biol. Chem.* **91**, 1.
 de Fremery (1931). *Acta Brev. Néerl.* **1**, 110.
 Freud, de Fremery and Laqueur (1932). *Pflüger's Arch.* **229**, 763.
 ——— Laqueur and Pompen (1932). *Endokrin.* **10**, 1.
 ——— de Jongh and Laqueur (1933). *Ned. Tijdsch. Gen.* **77**, 10.
 Gallagher and Koch (1930). *J. Pharm. Exp. Ther.* **40**, 327.
 Girard, Sandulesco, Fridenson and Rutgers (1932). *Compt. Rend. Acad. Sci.* **195**, 981.
 de Jongh (1930). *Arch. Néerl. Physiol.* **15**, 470.
 ——— Kober and Laqueur (1931). *Biochem. Z.* **240**, 247.
 Korenchevsky, Dennison and Kohn-Speyer (1933). *Biochem. J.* **27**, 557.
 Marrian (1930). *Biochem. J.* **24**, 435.
 Moore (1932). Sex and internal secretions, p. 340. (Ballière, Tindall and Cox, London.)
 ——— and Gallagher (1930). *J. Pharm. Exp. Ther.* **40**, 341.
 Voss and Loewe (1928, 1931). *Arch. exp. Path. Pharm.* **159**, 532.
 Wallen-Lawrence and van Dyke (1931). *J. Pharm. Exp. Ther.* **43**, 93.