The Action of Sex Hormones on Endometrial Secretion in Spayed Rats Sensitized to Oestradiol

Numerous studies, some of them dating back to the very beginning of the era of sex endocrinology, have been published dealing with the action exerted by sex hormones on the morphology of the endometrium. It is only comparatively recently, however, i.e. especially after the publication of HOMBURGER's work[1-4], that research has been undertaken to determine the changes which these hormones are capable of producing in endometrial secretion. The secretory function of the endometrium is today of major interest in connection with the analysis of the phenomena of reproduction; it is, in fact, endometrial secretion which determined the milieu confronting the spermatozoa prior to fecundation of the ovum as well as the blastocyst prior to implantation.

The present study is concerned with the effects of oestradiol, testosterone, and progesterone on certain properties of the fluid secreted by the endometrium of spayed adult rats sensitized to oestradiol.

The effects exerted by these hormones on the quantity, pH, and viscosity of the endometrial fluid have been correlated with the changes in the weight of the uterus occurring in response to the substances administered.

Material and methods. Adult female rats (Sprague-Dawley), each weighing between 160 and 250 g, were subjected to ovariectomy; at the same time, laparatomy was performed in order to close the distal ends of the uterine horns by electrocauterization, care being taken to leave the regional blood supply intact. In rats thus treated, the endometrial secretions accumulated in the uterus. Antibiotic treatment, consisting of 6000 U penicillin and 10 mg streptomycin daily, was given to each animal for 7 days by the s.c. route as a prophylactic measure against infection. All the animals were sensitized by treatment with oestradiol, given for 21 days in a dosage of 3 µg/kg daily s.c. The hormones under study were administered s.c. from the 14th to the 21st day; the oestradiol-sensitized controls received sesame oil.

The animals were sacrificed 24 h after completion of the treatment. Using sterile procedures, the uterine fluid was recovered and pooled from all the animals in the same group, after which 30 U penicillin and 0.5 mg streptomycin was added per millilitre. Periodical inspection of bacteriological cultures made it possible to check the sterility of the fluid, infected batches being discarded.

The weight of the uterus was measured before and after total aspiration of the fluid, the difference in the measurements indicating the quantity of fluid that had accumulated. The pH was determined on the surface of the endometrial epithelium, as well as in the uterine fluid itself, using a Polymetron type 39 C. Finally, the viscosity of the fluid, expressed in centipoises, was measured with the aid of a Hess microviscometer (laboratory model) calibrated by means of sucrose solutions of various concentrations.

Results (cf. Table). *Weight of the uterus.* The treatment given in addition to the oestradiol produced only a slight increase in the weight of the uterus as compared with the controls. Administered in high doses, testosterone caused a small increase in the weight of the uterus, this increase appeared to be of a transitory nature, amounting to $+168 \pm 25$ mg in response to 30 mg/kg, but to only $+88 \pm 23$ mg in response to 100 mg/kg. When given in the smallest dose, progesterone had no influence on the weight of the uterus, although it did produce a clear-cut decrease in the weight of the organ when administered in doses of 10 mg/kg and upwards (-100 mg ± 12 mg to -140 ± 14 mg).

Quantity of fluid in the uterus. The figures for the quantity of fluid in the uterus are expressed in milligrammes, i.e. corresponding to the difference in the weight of uterus before and after removal of the fluid; the average value recorded in the controls was 1729 mg. In the doses in which it was administered, oestradiol tended to produce a slight, but insignificant increase in the quantity of fluid. In doses of up to 30 mg/kg, testosterone had no appreciable effect, but it did considerably reduce the production of fluid (-1121 ± 124 mg) when employed in a dose of 100 mg/kg. Given in a dose of 3 mg/kg, progesterone caused a slight diminution in the quantity of fluid (-607 ± 123 mg); the decrease, however, became much more marked in response to 10 mg/kg (-1325 ± 73 mg), and even greater when higher doses were administered (-1526 ± 60 mg and -1586 ± 55 mg).

pH of the epithelial surface and of the uterine fluid. In the controls, the pH was 7.52 on the surface of the uterine mucosa and 8.36 in the uterine fluid. Replacement therapy with oestradiol produces no appreciable change in these values. When employed in high doses of 30 mg/kg and 100 mg/kg, testosterone slightly decreased the pH of the epithelium (7.09 and 6.96, respectively), but had very little effect on the pH of the uterine fluid. In response to progesterone, a distinct decrease was observed in the pH of the epithelium – a decrease which became progressively more marked between dosage levels of 10 mg/kg and 100 mg/kg. The same tendency towards acidification was found in the uterine fluid: at a dosage of 10 mg/kg it was barely detectable (8.06), but at a dosage of 30 mg/kg it became quite pronounced (7.01), and in response to 100 mg/kg it was very marked.

Viscosity of the uterine fluid. The uterine fluid from the control animals showed a low degree of viscosity (4.56 centipoises), which treatment with oestradiol had the effect of slightly increasing. Treatment with low doses of testosterone had no influence on the viscosity of the uterine fluid, whereas high doses caused a rapid increase in its viscosity. The same tendency was even more marked in the animals receiving progesterone: although progesterone had absolutely no effect in a dose of 3 mg/kg, doses of 10 mg/kg – and particularly doses higher than this – elicited a strong increase in the viscosity of the uterine secretions (30 mg/kg: 335 centipoises).

Discussion. The use of spayed animals sensitized to oestradiol, in preference to intact animals, made it possible in these experiments to study the direct action exercized by the hormones on the uterine targed organ and to eliminate any effects which might have been exerted on the regulation of ovarian secretion.

The changes in the weight of the uterus observed under the conditions of the experiments indicate that the doses of oestradiol employed tend slightly to enhance the stimulation produced by oestrogen sensitization. When given in large doses, testosterone shows this same action to a more marked degree. On the other hand, all the doses of progesterone administered exert an antagonistic

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Effects of sex hormones on the weight of the uterus and on the properties of the uterine fluid

Substances	Dose in mg/kg	No. of animals	Weight of empty uterus	Weight of uterine fluid	No. of animals	pH of endometrium	No. of animals	pH of uterine fluid	No. of animals	Viscosity of uterine fluid in centipoises
Controls	–	50	417 ± 12[a]	1729 ± 85	34	7.52 ± 0.04	14	8.35 ± 0.05	15	4.56 ± 0.57
Oestradiol	0.003	5	430 ± 20	1715 ± 141	5	7.10 ± 0.08	2	7.70	2	6.25
	0.01	12	444 ± 32	1937 ± 264	6	7.22 ± 0.12	3	8.17 ± 0.11	3	4.85 ± 1.13
	0.03	11	479 ± 28	2203 ± 237	5	7.33 ± 0.03	3	7.88 ± 0.19	3	7.23 ± 0.24
Testosterone	3	6	382 ± 34	1563 ± 306	6	7.33 ± 0.07	2	8.20	2	5.40
	10	8	535 ± 27	2212 ± 227	8	7.36 ± 0.05	2	8.18	2	3.50
	30	9	585 ± 39	1298 ± 191	9	7.09 ± 0.04	2	7.98	2	18.80
	100	7	505 ± 35	608 ± 164	7	6.96 ± 0.07	1	8.20	1	35.80
Progesterone	3	11	396 ± 38	1122 ± 162	7	7.46 ± 0.11	3	8.30 ± 0.11	3	4.87 ± 1.58
	10	27	312 ± 13	404 ± 61	18	7.00 ± 0.07	6	8.06 ± 0.23	5	282.60 ± 146
	30	25	276 ± 16	202 ± 36	22	6.81 ± 0.06	4	7.01 ± 0.54	2	335
	100	15	313 ± 14	143 ± 25	14	6.61 ± 0.03	2	5.60	1	2340

[a] Standard error based on the mean established for the average values corresponding to more than 2 individual values.

effect, i.e. they reduce the degree of stimulation provoked by oestrogen sensitization.

In line with the results reported by SHIH et al.[5], who analysed the chemical composition of the fluid accumulated in the rat uterus, this fluid may be considered as a product of secretion (a conclusion, however, which is not entirely shared by RINGLER[6]). The maintenance of fluid production in response to oestradiol, which in our experiments was observed in spayed rats, has also been reported in other animal species (HOMBURGER et al.[4]). However, whereas the decrease in liquid production observed after administration of testosterone tallies with the findings of HOMBURGER et al.[4], the much more marked inhibition seen in response to progesterone is at variance with the observations reported by these authors, who found, on the contrary, that when intact mice were treated with progesterone the volume of secretion increased and the secreted fluid became diluted. This discrepancy might possibly be attributable to differences either in specific sensitivity or in the doses administered or in the methods of assay.

According to BLANDAU et al.[7], the uterine fluid of the rat shows an alkaline reaction during oestrus; similar findings have been obtained by other authors[5,8] in the rat and rabbit. The results described in the present study indicate that the pH of the fluid is dependent to some extent on the influence of sex hormones. There was a marked tendency towards acidification to particularly in response to progesterone; we cannot, however, offer any explanation for this phenomenon.

The changes in the viscosity of the uterine fluid, and especially the increase in its viscosity produced by progesterone, are quite striking. The increase in the viscosity of the uterine fluid observed in these experiments would not appear to depend on the volume of the fluid. For example, although testosterone (100 mg/kg) and progesterone (10 mg/kg) have comparable effects on the quantity of fluid, the viscosity of the fluid seems to remain considerably lower after treatment with testosterone. It would be desirable to determine precisely what factors are modified in this response, i.e. whether it is the quantity of formed elements, the absolute concentration of the components as a whole, or, alternatively, the relative concentration of one component in particular.

The observations reported in this paper reveal that the properties exhibited by the fluid secreted from the endometrium are closely dependent on the prevailing hormonal conditions. The change in these properties occurring under treatment with steroid sex hormones might possibly be one of the factors responsible for the fertility-inhibiting action of such steroids.

Zusammenfassung. Ligatur der distalen Enden der Uterushörner von adulten, kastrierten, mit Östradiol sensibilisierten Ratten hat eine Ansammlung der uterinen Flüssigkeit zur Folge. Die Wirkung von Östradiol, Testosteron und Progesteron auf die Eigenschaften dieser Flüssigkeit wurde untersucht. Während Östradiol in verschiedenen Dosen keinen signifikanten Effekt aufweist, zeigt Testosteron und besonders Progesteron eine ausgeprägte Wirkung. Sie reduzieren die Menge der Flüssigkeit, erniedrigen das pH und steigern die Viskosität. Diese Veränderungen könnten für die Hemmung der Fertilität durch diese Substanzen von Bedeutung sein.

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