THE EFFECT OF MESTRANOL AND LYNOESTRENOL ON UTERINE HISTAMINE IN THE RAT

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Shelesnyak (1959) first suggested that histamine might be the inducing factor for decidualization and that it might be involved in the process of implantation in the rat (Shelesnyak & Kraicer, 1964). Treatment with oestrogen results in histamine depletion from the rat uterus (Szego, 1966; McKercher, Van Orden, Bhatnagar & Burke, 1973), and it was suggested that histamine might be involved in the hyperaemia and oedema stimulated by oestrogen (Szego, 1966). Recently, it has been reported that treatment with the contraceptive steroids, mestranol and lynoestrenol, alone or in combination, resulted in a decrease in histamine content per ovary in the rat (Abdel-Aziz, Ghazal & Daabees, 1974). The present work was undertaken to study the effect of mestranol and lynoestrenol on the histamine content of the rat uterus.

Fifty-six virgin female albino rats with a mean body weight of 112 ± 5 g were allocated equally to four groups. The animals selected showed regular oestrous cycles. The contraceptive steroids were administered orally and were suspended in 0.5% carboxymethylcellulose (CMC). Treatment was started when the animals were in natural oestrus and was continued for 12 consecutive days, the daily dose being suspended in 0.2 ml CMC. Animals in the first group received 0.08 mg mestranol/rat/day, in the second group 2.5 mg lynoestrenol/rat/day, in the third group 0.08 mg mestranol+2.5 mg lynoestrenol/rat/day and in the fourth group corresponding volumes of 0.5% CMC to serve as controls. Histamine extraction was carried out as described by Parrat & West (1957) and histamine bioassay was performed as described by Barsoum & Gaddum (1935). In each group, seven sets of pooled uteri, each from two animals, were extracted for histamine. The uterine histamine content was expressed in terms of μg histamine/g uterus and /uterus.

For microscopic examination, histological sections were prepared, and stained with haematoxylin and eosin. Unna's method (1968) was used for differential staining of mast cells. Mast cells were counted per section in the mesometrial triangle, the myometrium and the endometrium.

The effect of contraceptive steroids on uterine histamine is shown in Table 1. Treatment with mestranol alone or in combination with lynoestrenol

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Table 1. Effect of treatment with mestranol and lynoestrenol, alone and in combination, on rat uterine histamine

<i>Uterine histamine</i>							
<i>Controls</i>		<i>Mestranol</i>		<i>Lynoestrenol</i>		<i>Mestranol + lynoestrenol</i>	
$\mu\text{g/g}$	$\mu\text{g/uterus}$	$\mu\text{g/g}$	$\mu\text{g/uterus}$	$\mu\text{g/g}$	$\mu\text{g/uterus}$	$\mu\text{g/g}$	$\mu\text{g/uterus}$
2.82 ± 0.4	0.699 ± 0.06	1.71 $\pm 0.21^*$	0.542 $\pm 0.04^*$	1.79 $\pm 0.24^*$	0.441 $\pm 0.03^{**}$	1.56 $\pm 0.2^{**}$	0.429 $\pm 0.03^{**}$

The uterine histamine values are expressed as Mean $\pm$ S.E.M.

* $P < 0.05$; ** $P < 0.01$. The P values indicate the level of significance of differences between the means of control and treated animals. For number of rats and number of uteri, see text.

resulted in a significant increase in uterine weight in mg per 100 g body weight ($P < 0.05$), while lynoestrenol treatment resulted in a non-significant increase ($P > 0.05$). Treatment with mestranol and lynoestrenol alone or in combination resulted in a significant decrease in uterine histamine when expressed in terms of μg histamine/g uterine tissue or /uterus.

Uteri from rats treated with mestranol showed pronounced histological changes reflecting the oestrogenic effect of this steroid, while uteri from rats treated with lynoestrenol showed less marked oestrogenic manifestations. The combination of mestranol and lynoestrenol resulted in uterine histological changes which reflected the predominance of oestrogenic activity. The effect of contraceptive steroids on uterine mast cells is shown in Table 2. The greatest number of mast cells in the rat uterus occurred in the mesometrial triangle and in the myometrium near the blood vessels, with few cells in the endometrium. Treatment with contraceptive steroids decreased the number of mast cells in

Table 2. Effect of treatment with mestranol and lynoestrenol, alone and in combination, on mast cell counts in the different parts of the rat uterus

<i>Group</i>	<i>No. of mast cells</i>			
	<i>Mesometrial triangle</i>	<i>Myometrium</i>	<i>Endometrium</i>	<i>Total</i>
Control (12)	7.4 $\pm$ 1.20	7.3 $\pm$ 1.30	1.08 $\pm$ 0.34	15.8 $\pm$ 2.28
Mestranol (13)	4.7 $\pm$ 0.98 $P > 0.05$	6.5 $\pm$ 0.94 $P > 0.05$	0.31 $\pm$ 0.24 $P > 0.05$	11.5 $\pm$ 1.55 $P > 0.05$
Lynoestrenol (11)	3.2 $\pm$ 0.91 $P < 0.01$	5.3 $\pm$ 1.08 $P > 0.05$	0 $P < 0.01$	8.5 $\pm$ 1.84 $P < 0.01$
Mestranol + lynoestrenol (9)	5.7 $\pm$ 1.52 $P < 0.05$	9.0 $\pm$ 2.28 $P < 0.05$	1.00 $\pm$ 2.29 $P > 0.05$	15.7 $\pm$ 3.20 $P > 0.05$

The uterine mast cell counts are expressed as Means $\pm$ S.E.M. The P values indicate the level of significance of differences between the means of control and treated animals. The numbers in parentheses indicate the number of uteri examined.

the mesometrial triangle. This decrease was significant after treatment with lynoestrenol alone or in combination with mestranol, while mestranol treatment resulted in a non-significant decrease. The number of mast cells in the myometrium did not change significantly after any of the hormonal treatments. Only lynoestrenol treatment resulted in a significant decrease in the number of mast cells in the endometrium and also in the total number of mast cells in the uterus.

The reduction in uterine histamine following treatment with the synthetic oestrogen, mestranol, was parallel with that reported after treatment with natural oestrogen (Szego, 1966; McKercher *et al.*, 1973). It was not expected, however, that treatment with lynoestrenol, a dominant progestational steroid, would also result in reduction of uterine histamine. The weak oestrogenic activity reported for lynoestrenol (Overbeek, Madjerek & Divisser, 1962; Okada, Amatsu, Ishihara & Tokuda, 1964; Paulsen, 1965) has also been shown by the effect of this steroid on the vaginal smear (Abdel-Aziz *et al.*, 1974) and the histological changes in the uterus observed in the present work. The reduction in uterine histamine observed after treatment with lynoestrenol might thus be mediated through its oestrogenic action or through its progestational and oestrogenic actions.

It has recently been reported that treatment with oestradiol-17 β resulted in a reduction in uterine and 5-hydroxytryptamine which was consistent with a reduction in uterine mast cell count (McKercher *et al.*, 1973). In the present study, an attempt was made to investigate the effect of treatment with contraceptive steroids on uterine mast cells. Such treatment resulted in a reduction in the number of mast cells in the mesometrial triangle. Besides containing the highest number of mast cells, the mesometrial triangle may play an important rôle in the regional regulation of uterine vascularity and permeability (De Feo, 1967). In view of the evidence provided by Schayer (1962) concerning the rôle of histamine in regulation of the microcirculation, it is possible that histamine depletion in the uterus, particularly from the mesometrial triangle, following treatment with contraceptive steroids, may be one of the factors contributing to the antifertility action of these steroids in the rat. The significant reduction in uterine histamine observed after all hormonal treatment, but with a parallel significant decrease in the total cell count only after treatment with lynoestrenol, indicates that this decrease in uterine histamine may be mainly from non-mast cell sites.

REFERENCES

- ABDEL-AZIZ, A., GHAZAL, A. & DAABEES, T. (1974) The effect of mestranol and lynoestrenol on ovarian histamine in the rat. *J. Reprod. Fert.* **40**, 463-466.
- BARSOUM, G. S. & GADDUM, J. H. (1935) The pharmacological estimation of adenosine and histamine in blood. *J. Physiol., Lond.* **85**, 1-14.
- DE FEO, V. J. (1967) Decidualization. In *Cellular Biology of the Uterus*, pp. 191-290. Ed. R. M. Wynn. North-Holland Publishing Company, Amsterdam.
- McKERCHER, T. C., VAN ORDEN, L. S., III, BHATNAGAR, R. K. & BURKE, J. P. (1973) Estrogen-induced biogenic amine reduction in the rat uterus. *J. Pharmac. exp. Ther.* **185**, 514-522.
- OKADA, H., AMATSU, M., ISHIHARA, C. S. & TOKUDA, G. (1964) Conversion of some synthetic progestins to estrogens. *Acta endocr., Copenh.* **46**, 31-36.

- OVERBEEK, G. A., MADJEREK, Z. & DIVISSER, J. (1962) The effect of linoestrenol on animal reproduction. *Endocrinology*, **41**, 351–370.
- PARRAT, J. R. & WEST, G. B. (1957) 5-Hydroxytryptamine and mast cells. *J. Physiol., Lond.* **137**, 169–177.
- PAULSEN, C. A. (1965) Progesterone metabolism: special references to estrogenic pathways. *Metabolism*, **14**, 313–319.
- SCHAYER, R. W. (1962) Evidence that induced histamine is an intrinsic regulator of the microcirculatory system. *Am. J. Physiol.* **202**, 66–72.
- SHELESNYAK, M. C. (1959) Fall in uterine histamine associated with ovum implantation in pregnant rats. *Proc. Soc. exp. Biol. Med.* **100**, 380–381.
- SHELESNYAK, M. C. & KRAICER, P. F. (1964) Studies on the mechanism of nidation. XI. Duration of the inhibition of decidual induction by antihistamines. *J. Reprod. Fert.* **8**, 287–292.
- SZEGO, C. M. (1966) Role of histamine in mediation of hormone action. *Fedn Proc. Fedn Am. Socs exp. Biol.* **24**, 1343–1352.
- UNNA, P. G. (1968) *Manual of Histologic Staining Methods of the Armed Forces*, 3rd edn, p. 115. Institute of Pathology Publication, McGraw-Hill, New York.