The Effect of Estrone and Stilbestrol on the Response of Rabbits' Uteri to Ergonovine

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It is a common experience in the assaying of oxytocic drugs on isolated uteri of guinea pigs and rabbits that a considerable number of the organs have to be discarded because of their erratic responses. For example, if the animal is immature, the uterus invariably requires large doses for a contraction, and after two or three trials it fails to respond any further. On the other hand, a mature animal gives rise to a uterus that may be highly congested and exercises vigorous, spontaneous contractions. In both instances, the organ is not suitable for a quantitative estimation of active substances. Only by prolonged segregation of young females from males, and by further selection of the uterus upon sacrifice, can one succeed in making a satisfactory test. A relatively pale but fully developed pair of uterine horns, as a rule, is most preferable. The method is wasteful and costly.

Reynolds (1) has summarized the present knowledge on the mammalian uterus, particularly the rabbit's. He repeatedly emphasizes the fact that the uterus becomes motile and irritable when it is under the influence of estrogenic substances, and that its responsiveness to posterior pituitary extracts is high if it has high irritability. It was based upon this principle that the present series of experiments was undertaken. The proposal of assaying ergonovine, a new ergot alkaloid, on the isolated rabbit's uterus was made by Swanson, Hargreaves and Chen (2). It occurred to us that if immature female rabbits were treated with estrone or stilbestrol, their uteri might be relatively more irritable and thus more responsive to ergonovine, just as they would be to pituitary extracts.

EXPERIMENTAL

To test this point, 159 New Zealand red rabbits, 6 of which were ovariectomized, were injected subcutaneously with various doses of estrone. Another group of 25 rabbits was similarly treated with stilbestrol. A third group of 10 animals was used as the control. In order to rule out any difference of reaction due to the strain of the animal, 20 albino rabbits were given estrone by subcutaneous injection. All animals weighed between 1.5 and 1.8 Kg. Both estrone and stilbestrol were dissolved in cottonseed oil, the concentration being 1:100,000 each. The total dose of either drug as listed in Table I was divided into six fractions, and one fraction was injected every second day. Two days after the last injection, namely, on the thirteenth day, the rabbit was scarified, and its uterus removed. A segment of the ovarian end was immersed in 100 cc. of Tyrode's solution kept at 37.5° C., and arranged for recording in precisely the same manner as the U. S. P. directs for the assay of posterior pituitary solution (3).

Table I.—Ergonovine Assay on Isolated Uteri of Rabbits Treated with Estrone and Stilbestrol

Number of Rabbits Used	Pre-Assay Medication: Drug	Pre-Assay Medication: Total Dose, γ per Animal	Uteri Suitable for Assay: Number	Uteri Suitable for Assay: Per Cent
10 (Control)	None	..	0	0
10	Estrone	3	0	0
10		6	0	0
20		9	15	75
45		12	42	93.3
28		15	25	89.3
10		18	6	60
10		24	5	50
10		30	6	60
10		60	4	40
5[a]	Estrone	6	0	0
5[a]		9	3	60
5[a]		12	4	80
5[a]		15	4	80
6[b]	Estrone	12	5	83.3
5	Stilbestrol	3	0	0
5		6	2	40
5		9	5	100
5		12	4	80
5		15	3	60

[a] White color.
[b] Ovariectomized.

After the muscle was completely relaxed, a dose of 1 cc. of 1:10,000 solution of ergonovine maleate was applied. Following a wash-out and a rest of 15 minutes, the dose was repeated. If the response matched that of the first dose, smaller amounts,

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equivalent to $^4/_5$, $^3/_5$ or less, were then tried. The muscle would be considered satisfactory provided the heights of contractions showed a quantitative relationship with the doses employed. An example is well illustrated in Fig. 1. It must be emphasized that the height of contraction (peak) is the only criterion, and that the same dose of ergonovine makes the muscle repeat its response with exactness.

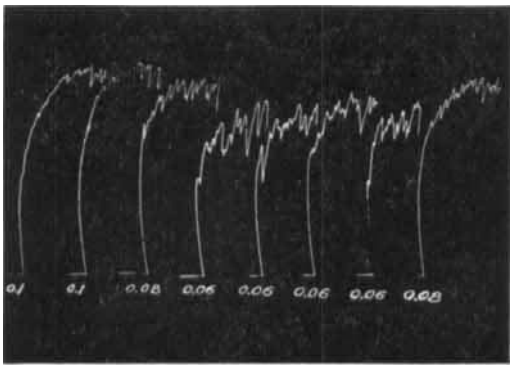

Fig. 1.—An Example of a Utilizable Strip of the Isolated Rabbit's Uterus.

The numbers indicate doses of ergonovine maleate in milligrams. Note the quantitative relationship with the amounts of the drug applied.

DISCUSSION

As shown in Table I, the results conclusively prove that subcutaneous injection of estrone or stilbestrol renders the immature rabbit's uterus more irritable, more reliable in response, and thus more utilizable in the assay of ergonovine. The optimal dose of estrone per animal is obviously in the neighborhood of 12γ, and that of stilbestrol, 9γ, each divided into 6 portions and injected in 11 days. Ovariectomy did not nullify the estrone action for 83% of the uteri was fully reactive. Although the white rabbits appeared slightly less benefited, the number of satisfactory preparations reached 80%. This is in strong contrast with the 10 control rabbits, none of which had a uterus suitable for assaying purposes. The economic advantage of the estrogenic therapy in rabbits cannot therefore, be overestimated. It is also probable that the ergonovine assay is attended by greater precision.

SUMMARY

1. Immature rabbits injected subcutaneously with divided doses of estrone or stilbestrol give rise to an overwhelmingly high percentage of uteri, which, when isolated, are suitable for ergonovine assays.

2. The optimal dose (total) of estrone is approximately 12γ per animal, and that of stilbestrol, 9γ.

3. The chief advantage of this procedure is economy. In addition, the assay may possibly be carried out with higher precision.

REFERENCES

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(2) Swanson, E. E., Hargreaves, C. C., and Chen, K. K., JOUR. A. PH. A., 24 (1935), 835.

(3) "United States Pharmacopœia XI" (1936), p. 217.

Assay Methods for Phenothiazine Pharmaceuticals*

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Phenothiazine has recently assumed an important role in the veterinary pharmaceutical field since it has been found to be a very efficient anthelmintic with a comparatively low toxicity (1). To date there are no published assay methods for determination of phenothiazine purity or of phenothiazine content in pharmaceuticals. Determinations of sulfur or nitrogen by standard methods were found to be unsatisfactory and, so far, colorimetric methods have been of no value. This report describes a method for the routine control assay of phenothiazine in pharmaceutical preparations, based on the extraction and subsequent weighing of the drug. Two principal types of pheno-

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