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Intrasplenic Implantation of Multiple Pellets of Estrogenic Steroids.

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When pellets of estrone, α-estradiol, and α-estradiol-3-benzoate are implanted intrasplenically in spayed female rats either no estrus or a very short period of estrus results (Biskind and Mark,[1] Biskind[2]). On the basis of the results which follow subcutaneous transplantation of the spleen containing the pellet, it was believed that α-estradiol-3-benzoate is inactivated by the liver to a lesser extent than is α-estradiol (Biskind[2]). This difference is only apparent. Pellet implantation fails to take into account the many variations in peripheral activity (Segaloff[3]).

The present study was undertaken to ascertain whether the differences in the observations of Biskind and ourselves could be explained by employing multiple pellets and pellets diluted with cholesterol (Shimkin and White[4]). In addition, we have employed the implantation of multiple pellets in order to study those estrogens which are too insoluble for satisfactory intrasplenic injection studies.

Material and Methods. Young adult spayed female rats of the Sprague-Dawley strain were employed for this study. Pellets were prepared in a simple die and compressed by means of a hand arbor press. The pellets were 2 mm in diameter and weighed from 4.0 to 6.0 mg when implanted. When necessary transplantations of the spleen were performed according to our previously reported technic (Segaloff[3]) before implantation of pellets. The pellets were implanted in a previously prepared small pocket in the spleen which was then closed with a single stitch. Only one pellet was implanted at any one time. Additional pellets were implanted as necessary until either 28 continuous days of estrus were induced or a maximum of 10 pellets per animal had been implanted. Vaginal smears were made daily at approximately the same time from castration until the animals were sacrificed.

Results. The results are presented in Table I.

All of the estrogenic materials studied and listed in Table I were also implanted intrasplenically into spayed female rats with subcutaneous spleens divorced from the portal circulation. The data on these latter animals are not presented in tabular form since the pellets were absorbed at about the same rate as those in the spleen *in situ* and one pellet only of each compound either alone or diluted with 3 parts of cholesterol sufficed to produce the standard 28-day period of estrus.

From Table I it is apparent that a single pellet of ethinyl estradiol, estriol, α-estradiol-3-benzoate, or α-estradiol dipropionate sufficed to produce 28 continuous days of estrus even when implanted into the spleen *in situ*. Accordingly, pellets of these compounds diluted with 75% cholesterol were studied with the results seen in Table I. Only minute amounts appear to be absorbed from the estrogen-cholesterol pellets. For the purpose of tabulation, the assumption was made that all of the loss in weight was due to the absorption of the estrogen. The assumption is not wholly valid. Fuenzalida[5] has observed that in pellets of steroid in combination with cholesterol, the absorption is not selective, and cholesterol is absorbed as well as steroid. Thus, from the data on estrogen-cholesterol pellets we must conclude that ethinyl estradiol, estriol, α-estradiol-3-benzoate, and α-estradiol

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1 Biskind, G. R., and Mark, J., *Bull. Johns Hopkins Hosp.*, 1939, **65**, 212.

2 Biskind, G. R., *Endocrinol.*, 1941, **28**, 894.

3 Segaloff, A., *Endocrinol.*, 1943, **33**, 209.

4 Shimkin, M. B., and White, J., *Endocrinol.*, 1941, **29**, 1020.

5 Fuenzalida, F., *Rev. Canad. de Biol.*, 1944, **3**, 366.

TABLE I.
The Response of Spayed Female Rats to Intrasplenic Estrogen Pellets.

	No. of rats	Avg No. pellets	Avg abs./ pellet/day μg	Avg total abs. per/day for 28 days estrus μg
1. Ethinyl estradiol	5	1	99	99
25% Ethinyl estradiol, 75% cholesterol	5	3.8	2	7
2. Estriol	4	1	26	26
25% Estriol, 75% cholesterol	4	5.3	2	9
3. α-Estradiol-3-benzoate	5	1	13	13
25% α-Estradiol-3-benzoate, 75% cholesterol	5	7.3	2	13
4. α-Estradiol dipropionate	5	1	39	39
25% α-Estradiol dipropionate, 75% cholesterol	5	4.2	4	17
5. Estrone-3-benzoate	5	4.2	6	26
6. α-Estradiol	6	2	17	35
7. Equilin	3	2.3	20	47
8. Estrone	5*	6	11	68
9. Equilenin	4	6.5	24	156

* One animal failed to display 28 days of estrus even after 10 pellets; this animal is omitted from the summary.

dipropionate are extremely active when administered by the intrasplenic implantation of pellets containing cholesterol, but due to the minute amounts absorbed no conclusion can be drawn as to their relative activity.

The remaining estrogens can be placed in the following descending order of activity as intrasplenically implanted pellets of the crystalline estrogen. Estrone-3-benzoate > α-estradiol > equilin > estrone > equilenin.

Discussion. The data presented confirms the reports that the intrasplenic implantation of a single pellet of many estrogens does not suffice to produce a prolonged period of vaginal estrus. However, the above data on the multiple implantations of pellets would indicate that the production of prolonged estrus is a function of the amount of estrogen absorbed and the character of the estrogen. This is in agreement with the results obtained with intrasplenic injections. Unfortunately, as previously pointed out, the method of pellet implantation in the subcutaneous spleen is too crude a method to enable one to take into account the obvious differences in the peripheral activity of such estrogenic compounds.

In addition, it is important to note that estriol which was not soluble enough for adequate study by intrasplenic injection, is but slightly inactivated by passage through the portal system. Equilenin, on the other hand, which was also not sufficiently soluble for adequate study by intrasplenic injection, apparently is inactivated in the liver to a marked degree.

Summary. If a sufficient number of estrogen pellets are implanted intrasplenically in spayed female rats, prolonged periods of estrous are produced. The number of pellets required depends upon the relative activity of the estrogen and its rate of absorption.

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