relation to its nitrogen content is given by the fact that a 1:1000 dilution of a solution containing 0.3 mg of nitrogen/cm^3 still retained skin reactivity in a ragweed-sensitive case. That is, a solution containing 0.0003 mg of nitrogen/cm^3 scratched into the skin by the usual technic, gave a positive test.

The skin reactivity of the pigmented fractions has not as yet been investigated nor have undialyzed solutions been examined electrophoretically.

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Absorption Rates and Biologic Effects of Pellets of α-Estradiol and α-Estradiol Benzoate in Women.

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In a preliminary communication, we have reported the subcutaneous implantation of crystals of α-estradiol benzoate in a group of 10 menopausal patients who had well-defined morphologic signs and symptoms of estrogen deficiency.[1] It was shown that, by this method of administering estrogens, it was possible to achieve a strikingly more prolonged effect than is obtained with comparable amounts of the hormone, administered parenterally, in solution in oil. It was subsequently demonstrated that a correspondingly prolonged inhibition of the hyperactive hypophysis occurred following the estrogen implantation.[2]

Since Deanesley and Parkes[3, 4] have shown that prolonged estrogenic effects resulted from the subcutaneous implantation of pellets of estrogens in rats, we thought it desirable to study in women the duration of the physiologic and therapeutic effects of pellets as compared with crystals of the same estrogenic substance. Accordingly, 46 patients were implanted with pellets and 55 with crystals of either α-estradiol or α-estradiol benzoate. During a period of observation of approximately one year, it was noted that more prolonged physiologic and therapeutic effects re-

1 Salmon, U. J., Walter, R. I., and Geist, S. H., *Science*, 1939, **90**, 162.

2 Salmon, U. J., Geist, S. H., and Walter, R. I., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 424.

3 Deanesley, R., and Parkes, A. S., *Proc. Roy. Soc. B.*, 1937, **124**, 279.

4 Deanesley, R., and Parkes, A. S., *Lancet*, 1938, **2**, 606.

sulted from the implanted crystals than from pellets of similar weight and chemical constitution. We, therefore, felt it important to determine the rate of absorption of the hormone by removing and reweighing the implanted pellets at varying intervals after the implantation. At the same time, the duration of biologic effects of the implanted hormone, as manifested by morphologic changes in the endometrium and vaginal mucosa, were studied by means of repeated vaginal smears and vaginal and endometrial biopsies. Here we wish to report the results of our studies on the absorption rate and duration of biologic effects of the implanted estrogen pellets.

Material and Procedure. From the 46 cases implanted with pellets, 14 patients (9 natural menopause, 4 surgical castrates and 1 X-ray castrate) were selected for excision. The duration of the menopause, in this group, varied from 2 months to 7 years. All of the patients had either clinical or morphologic evidence of estrogen deficiency, or both, prior to the implantation.

Round, flat pellets of α-estradiol and α-estradiol benzoate,* sterilized by autoclaving (265°F, at 15 lb pressure, for 30 minutes), varying in weight from 15 to 25 mg each, were implanted, subcutaneously, in the outer aspect of the thigh. Nine patients were implanted with a single pellet; 4 with 2 pellets; and one with 3 pellets. In 8 cases, the pellets were of α-estradiol and in 6, α-estradiol benzoate. The skin was prepared with alcohol and iodine and anesthetized with 1% novocaine. The pellets were implanted, approximately ¾ of an inch below the surface into the subcutaneous fat, through a skin incision approximately ½ inch in length. The implantation sites were excised at varying intervals after the implantation and the pellets were weighed after drying in a desiccator.

Results. Absorption Rates of α-Estradiol Pellets. The α-estradiol pellets were excised at periods of time varying from 130 to 245 days following the implantation. Each pellet was found to be closely enveloped by a fibrous capsule. Microscopic study of the surrounding tissues revealed a typical non-specific foreign body reaction. (The histologic details of the tissue reaction to the pellets are described elsewhere.[5]) The results of this study are presented in Table I.

The rate of absorption, expressed in terms of average percent

* For the α-estradiol and α-estradiol benzoate pellets used in this investigation, we are indebted to Dr. Erwin Schwenk, Schering Corporation, Bloomfield, N. J., and to Mr. Robert C. Mautner, Ciba Pharmaceutical Products, Summit, N. J.

[5] Geist, S. H., Walter, R. I., and Salmon, U. J., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 712.

TABLE I.

Case	Age	Menopause	Post-menopause, mo	Estrogen	Initial wt, mg	Wt on removal, mg	Duration of implantation, days	Amount absorbed, mg	Absorption per 30 days, %	Avg daily No. of rat units absorbed, R.U.	Maximum duration of therapeutic effect	Clinical status at excision
1	45	n	7	α-est	25	18.6	193	6.4	3.9	398	138 days	75% recurrence
2	50	n	36	"	15	11.0	160	4.0	5.0	300	160 plus	No "
3	47	n	24	"	45*	42.2	23	2.8	8.0	1461	0	No improvement
4	42	s	6	"	15	12.0	130	3.0	4.6	277	130 plus	No recurrence
5	45	n	36	"	25	16.4	180	8.6	5.7	573	134 days	75% "
6	46	n	13	"	15	9.6	213	5.4	5.1	304	0	No improvement
7	49	n	60	"	25	21.1	245	3.9	1.9	191	101 "	100% recurrence
8	48	n	3	"	15	9.8	225	5.2	4.6§	277¶	190 "	75% "
9	51	s	84	α-est-b	50†	49.4	87	0.6	0.41	41	0	No improvement
10‡	27	s	4	"	25	20.7	207	4.3	2.5	125	75 "	100% recurrence
					50†	48.0	101	2.0	1.2	119	60 "	100% "
11	51	n	60	"	25	20.4	116	4.6	4.7	238	62 "	100% "
12	50	n	24	"	50†	48.8	88	1.2	0.81	82	88 plus	No "
13	48	x	24	"	25	24.0	116	1.0	1.0	52	0	No improvement
14	38	s	2	"	50†	46.4	156	3.6	1.4**	138††	45 days	100% recurrence

* 3 pellets.
† 2 "
‡ This patient was implanted at 2 different times.
§ Average of α-estradiol series = 4.85% per 30 days.
** Average of α-estradiol benzoate series = 1.72% per 30 days.
¶ Average of α-estradiol series = 473 R.U. per day.
†† Average of α-estradiol benzoate series = 113 R.U. per day.
n = natural menopause.
s = surgical menopause.
x = x-ray menopause.
α-est = α-estradiol.
α-est-b = α-estradiol benzoate.

weight loss per 30 days, varied from 1.9 to 8, with an average for the series of 4.85%. This represents, in terms of rat units, average daily absorption rates varying from 191 to 1460 R.U., with an average for the series of 473 R.U. per day. Deanesley and Parkes, in their study of α-estradiol pellets in rats, reported 6% to 9% average absorption per month.

Absorption Rates of α-Estradiol Benzoate Pellets. The α-estradiol benzoate pellets were excised at intervals varying from 87 to 207 days after the implantation. In this series, also, a fibrous capsule was found surrounding each pellet. The rate of absorption in terms of average percent weight loss per 30 days, varied from 0.41 to 4.7. The average rate of absorption (per 30 days), of this series, was 1.72%. This represents, in terms of rat units, variations from 41 to 238 R.U. per day, with an average for the series of 113 R.U. per day.

Biologic Effects of α-Estradiol Pellets. All cases showed characteristic morphologic evidence of estrogenic effect, as indicated by proliferative response in the vaginal mucous membrane (smears and biopsies) and/or the endometrium, within 2 weeks after the implantation. Details of the effect of implanted estrogens upon the mucous membranes of the genital tract are being reported elsewhere.

At the time of removal of the implanted α-estradiol pellets, there was evidence (in all but 3 cases) of morphologic regression to the pre-implantation status, indicating varying degrees of estrogen deficiency. Apparently, at the time of excision, so little of the hormone was being absorbed that no estrogenic effect was demonstrable in the uterine or vaginal mucosa. In all of these cases the pellets had been retained for 180 days or more.

The 3 cases in this series that revealed morphologic evidence of continued estrogen activity, at the time of excision, had all been implanted for shorter periods of time (23, 130 and 160 days).

Biologic Effects of α-Estradiol Benzoate Pellets. In the α-estradiol benzoate series, morphologic studies, at the time of excision (87 to 207 days post-implantation), revealed, in all cases, regression to the pre-implantation status, indicating the cessation of estrogen activity.

Clinical Effects of α-estradiol Pellets. One patient (Case No. 6) experienced no relief of symptoms; 4 were relieved for periods varying from 101 to 190 days; 2 (Case No. 2 and Case No. 4) were still symptom-free at the time of excision (160 and 130 days post-implantation).

Clinical Effects of α-Estradiol Benzoate Pellets. Two patients (Case No. 9 and Case No. 13) experienced no relief of symptoms following the implantation; 5 were relieved for periods varying

from 45 to 75 days; one (Case No. 12) was symptom-free at the time of excision, which was 88 days after the implantation. It is apparent from this study that pellets of α-estradiol have a more prolonged therapeutic and biologic effect than pellets of α-estradiol benzoate.

It is important to note that despite the presence of sizeable pellets (weighing 11 to 46.4 mg) in the subcutaneous tissue, the majority of the patients, at the time of excision, exhibited clinical as well as morphologic evidence of estrogen deficiency. The fact has already been mentioned that the pellets were found to be completely enveloped by a tight, fibrous capsule.[5] Apparently the capsule acts as a barrier, progressively retarding absorption of the hormone and reducing it finally to a level at which no demonstrable estrogen effect is exerted, either clinically or morphologically.

The question may be raised as to whether the hormone may not be inactivated by its prolonged contact with the subcutaneous tissues. Such a qualitative change in the pellets has been considered and apparently ruled out by demonstrating that the excised pellets, when reimplanted in rats, produced characteristic estrogen effects.

It is evident from this study that, in spite of the striking initial morphologic and therapeutic effects produced by the implanted estrogen pellets, there is a serious objection to this method of administering estrogens clinically, since the therapeutic effect is relatively short-lived and the patients, thereafter, retain sizeable pellets without deriving any therapeutic benefit from them.

Summary and Conclusions. A comparative study of the absorption rates and duration of biologic effectiveness of implanted pellets of α-estradiol and α-estradiol benzoate was made in a series of 14 cases. This study revealed that the average percent absorption rate, per 30 days, of α-estradiol pellets was 4.85% (= 473 rat units per day), as compared to 1.72% (= 113 rat units per day) for pellets of α-estradiol benzoate. The duration of biologic and therapeutic effects was definitely longer in the α-estradiol series. It is concluded, on the basis of these studies, that the fibrous capsule which forms about the pellets progressively decreases the rate of absorption of the hormone, so little being absorbed finally that no demonstrable morphologic or therapeutic effect is produced. Furthermore, because of the fact that absorption of the hormone in effective amounts ceases when only a relatively small amount of the pellet has been absorbed, it is concluded that the implantation of pellets (weighing 15 to 25 mg) of α-estradiol and α-estradiol benzoate is not a satisfactory method of administering estrogens clinically.

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