2. This amount did not affect blood sugar and serum inorganic phosphorus changes during glucose tolerance tests and insulin tolerance tests in the same subjects. 3. Since these amounts of D.B.I. when continued on daily dosage do induce hypoglycemia, it is evident that such responses result either from accumulation of the drug in body fluids and tissues, represent acquired responsiveness upon continued exposure, or both.

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Duration of Progestational Effect in Rabbit Endometrium after Prolonged Administration of Progestogens.* (25345)

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Since the study by De Fremery, Luchs and Tausk(1), it has generally been assumed that progestative effect of progesterone on rabbit endometrium cannot be extended beyond 17 days. This could be explained by interaction of 3 factors: 1) lack of response of endometrium after 17 days; 2) lack of estrogen, the primary effect of which is considered necessary for progestative transformation and its continuity; 3) suppression of pituitary gonadotropins or a similar factor which might stimulate endogenous estrogen or progesterone formation. The complexity of the factors involved led us(1) to assume the existence of an "inhibitory mechanism which needs further elucidation." This paper is devoted to the above problem.

Technic. Experiments were carried out on 200 female rabbits weighing approximately 1,000 g each. They were primed by daily injections of 15 μg estrone (in 0.15 ml olive oil) for 6 days. After 1 day's rest, the progestational compounds were injected subcutaneously at dosage level of 0.5 mg (in 0.05 ml olive oil) daily. The progestational effect was assessed according to McPhail(2) after sacrificing rabbits within 1-80 days after commencement of injection of the progestational substance. At autopsy, 2 segments were removed from each horn and preserved in 4% formalin (1:10) for histological examination. Evaluation of progestational transformation was carried out according to McPhail(2), the sections of uterus scored as 0, 1, 2, 3, 4 respectively. Furthermore, ovaries were inspected to ensure that no corpora lutea were present. We did not use the technic of De Fremery, Luchs and Tausk(1) who followed progestational transformation in each animal by laparotomy and removal of a piece of uterine horn at different time intervals, lest it interfere with degree and continuity of progestational transformation.

Results. A. *Progesterone.* Table I shows that, during 80 days' treatment with progesterone, secondary transformation of endometrium could be obtained. We believe that this is possible after longer intervals; thus, in

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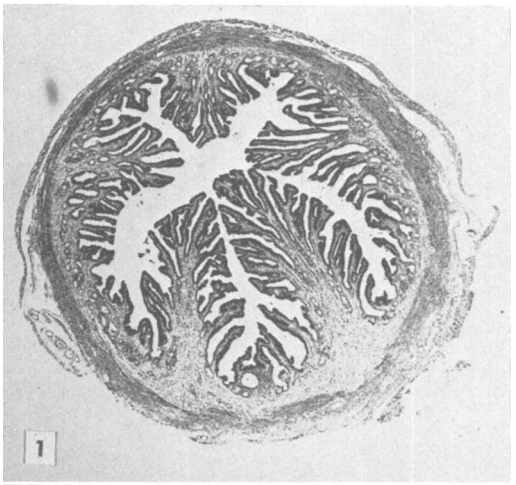

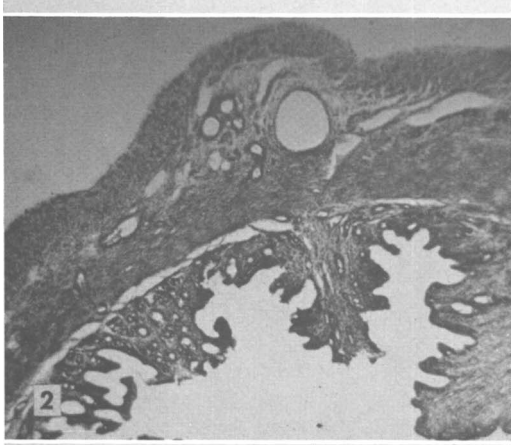

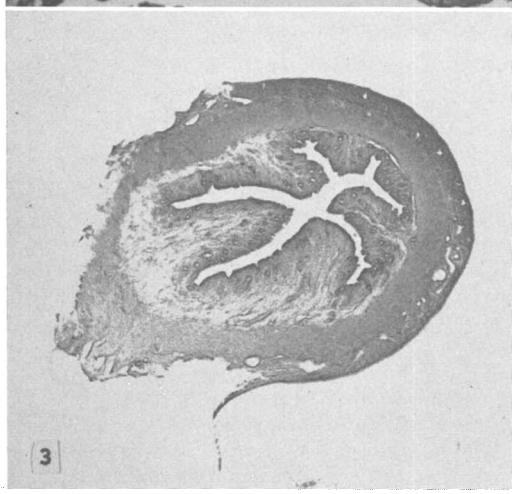

FIG. 1. Rabbit uterus (25×). Progestational transformation after 96 days' treatment.

FIG. 2. Uterus of rabbit on 17th day of protracted treatment with 0.5 mg/day progesterone (40×). Note decay of progestational phase.

FIG. 3. Uterus of rabbit on 34th day of protracted treatment with 0.5 mg/day progesterone (30×). Note absence of progestational transformation.

one rabbit treated for 96 days, degree 4 of progestational transformation was found (Fig. 1).

Table I shows, however, that at certain periods there appear negative or low score progestative phases. These periods are 15th-19th day and multiples thereof, *i.e.*, 29th-35th day, etc. This can only be explained by the breaking down of progestational phase at these terms and its immediate restoration.

We studied this problem in 2 ways: 1) Vaginal smears were full of erythrocytes and debris during days of progestational breakdown, whereas during remaining time they showed an epithelial pattern. In the former, the uterus showed decay of the progestational phase (Fig. 2). In Fig. 3 the transient proliferative stage, assumed to occur on day after vaginal bleeding, is presented. This photograph was taken from uterus of a rabbit on 34th day of protracted progesterone treatment.

2) We also studied how long the endometrial breakdown could last, and how soon the progestational phase could be restored. The vaginal smears continued to show erythrocytes and debris only during *2* consecutive days. As a rule we obtained the progestational phase in estrogen-primed rabbits as early as *2* days after injection of progesterone (*cf.* Table I). It can thus be safely said that during protracted progesterone treatment a breakdown of progestational endometrium occurs on 17th ± *2* day, followed within *2* days by building up of a new progestational endometrium.

B. *New Progestogens.* We tested the following new progestogenic substances: 17-alpha-hydroxy-progesterone caproate, norethindrone, methallyl-nortestosterone, at 0.5 mg/day. After estrogen-priming rabbits received the different progestogens for 20, 40, 60 and 80 days, respectively. At intervals of 17 ± *2* days vaginal smears showed erythrocytes and debris, indicating breakthrough bleeding. In all cases a progestational endometrium was found at necropsy. An endometrium of 60th

TABLE I. Progestational Effect in Rabbits after 1-80 Days' Subcutaneous Treatment with 0.5 mg Progesterone.

Days of treatment	Progestational scoring	Days of treatment	Progestational scoring	Days of treatment	Progestational scoring	Days of treatment	Progestational scoring
1	0, 0	21	2	41	3, 3	61	0, 0
2	3, 4	22	1, 3	42	3, 3	62	0, 1
3	4	23	1, 2, 2, 2, 3	43	3, 4	63	0, 2
4	4	24	1, 3	44	4, 4	64	0, 1, 2
5	4	25	1, 3	45	0, 0, 1	65	0, 2
6	4	26	2, 4	46	0, 0, 1, 1	66	0, 4
7	4	27	3, 4	47	0, 0, 1	67	0, 2
8	4	28	4, 4	48	0, 0, 2	68	0, 3
9	4	29	0, 0, 1, 1, 2	49	0, 1, 1	69	2, 4
10	4	30	0, 1, 1, 1	50	0, 1, 1	70	4, 4
11	4	31	0, 0, 1, 1	51	0, 0, 1, 4	71	3, 4
12	3	32	0, 0, 1, 2	52	2, 3	72	4, 4
13	4, 2	33	0, 0, 1, 2	53	2, 4	73	4, 4
14	4, 4	34	0, 0, 1, 3	54	3, 4	74	1, 2
15	0, 1, 2, 3	35	0, 0, 1, 2	55	4, 4, 4	75	0, 4
16	0, 1, 2, 4	36	3, 4	56	3, 4	76	0, 1
17	0, 1, 3, 3	37	2, 2	57	2, 3, 4	77	0, 2
18	2, 3, 3, 3	38	2, 4	58	4, 4	78	0, 1
19	2, 3, 3	39	3, 4	59	2, 4	79	0, 3
20	4, 4	40	2, 2	60	0, 1, 2	80	4, 4

day showed beginning of breakthrough bleeding as expected.

Discussion. Our results confirm reports of earlier investigators(1) that the progestative phase of rabbit endometrium breaks down after 17 days in spite of continued treatment. On the other hand, they reveal the almost forgotten fact that in the rabbit transformation to the second generative phase is accomplished within 48 hours. Therefore the progestational phase which breaks down near the 17th $\pm$ 2 day is restored within 48 hours when treatment with progestogens is continued. This would mean that through protracted treatment with progestogens, rabbit endometrium can be kept indefinitely in the second phase.

Junkmann(3,4) also noted waning of the progestational effect of a single injection of 20 mg hydroxy-progesterone caproate between 15th and 19th day. He believed that daily addition of 0.05 μg estradiol prolonged the progestational effect.

The progestational effect obtained by protracted treatment of rabbits with chorionic gonadotropin is likewise restricted to about 17 days' duration(5). By resuming gonadotropin treatment beyond this term it is, however, possible to obtain a quick return of the second phase(6,7). The mechanism of this phenomenon was studied by Zondek(8,9) who found that breakthrough bleeding might be obtained even after 5 days' treatment and that it closely resembled human menstruation.

In women the problem is different, as breakthrough bleeding occurs with progesterone(10, 11) and with most of the new progestogens. Greenblatt *et al.*,(12,13) delayed menses for as long as 6-7 months with 20-30 mg norethisterone daily. Large doses (up to 1,000 mg/day) of ethisterone, 17-acetoxyprogesterone, 17-hydroxyprogesterone caproate, could not prevent breakthrough bleeding. Boschann (14) delayed breakthrough bleeding until the 28th-42nd day of treatment with 100-200 mg/day ethinyl nortestosterone. Similar results are obtainable with medroxy-progesterone acetate. Following protracted treatment with chorionic gonadotropin, breakthrough bleeding occurs also between 17th and 19th day(15).

Summary. Continued injection of rabbits with progesterone, 17-alpha-hydroxy-progesterone caproate, norethindrone and methallyl-nortestosterone, maintained a progestative phase for 80 days and longer. At intervals of 17 $\pm$ 2 days, however, there occurred breakthrough bleeding followed by renewal of second phase within 2 days.

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Effect of Sr^{89} on Fluoride Retention in the Rat.* (25346)

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Increasing numbers of communities using fluoridated water along with the increasing amount of radioactive strontium in the atmosphere have caused considerable speculation whether the mutual presence of fluoride and strontium increases retention of each within the body(1). The purpose of this study is to investigate retention of fluoride and radioactive strontium when both are fed simultaneously in the rat.

Method. One mc of Sr^{89} (as strontium chloride) was diluted to one liter with fluoride-free redistilled water. This stock solution was used throughout the 3 series of experiments. Series I. A total of 24 male Sprague-Dawley rats was divided according to initial body weight into 4 groups of 6 animals each. All animals received a low-fluoride diet (0.05 μg F/g) and fluoride-free redistilled drinking water *ad lib.*, and were housed in an air-conditioned room in individual stainless steel metabolism cages. Animals in Group A served as controls and received no additional supplement. Those in Group B received a single dose of 0.5 ml of Sr^{89} stock solution (501,600 cpm/ml) by stomach tube. Animals in Group C received 0.15 mg F (as NaF) daily by stomach tube, and those in Group D received both a single initial dose of 0.5 ml Sr^{89} and 0.15 mg F daily. Duration of experiment was 10 days. Urine and feces samples were collected separately throughout each 24-hour period. One-half of the urine and feces samples, was analyzed for F by methods previously described (2). The remaining half was analyzed for strontium by charring samples under infrared lamps and ashing for 6 hours in muffle furnace at 600°C. The ash was dissolved in 2 ml 0.1 N HCl, transferred to volumetric flask and diluted with redistilled fluoride-free water to 10 ml. A 1 ml aliquot was placed in an aluminum planchet and evaporated to dryness at 45°C and activity determined with a scintillation counter. Carcasses and femurs were analyzed for F by methods previously described(2). To determine strontium content in calcified tissues, an aliquot of ash was placed in aluminum planchets, dissolved in 2 ml 0.1 N HCl, evaporated to dryness, and the activity counted. Series II was identical to Series I except for amounts of supplemental F and Sr^{89} (Table III) and duration of experiment (20 days). Series III was similar to Series I and II except that its duration was 100 days and supplements were different (Table V).

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