either anti-9 or anti-12 sera and used to immunize rabbits. The antisera obtained contained both 9 and 12 antibodies indicating that the 9 and 12 antigens of *S. typhosa* are on the same molecule.

1. Whiteside, R. E., Baker, E. E., *J. Immunol.*, in press.
2. Cohen, H. H., *ibid.*, 1958, v81, 445.
3. Webster, M. E., Sagin, J. F., Landy, M., Johnson, A. G., *ibid.*, 1955, v74, 455.
4. Staub, A. M., Pon, G., *Ann. Inst. Pasteur*, 1956, v90, 441.
5. Leskowitz, S., Waksman, B., *J. Immunol.*, 1960, v84, 58.
6. Terres, G., Wolins, W., *ibid.*, 1961, v86, 361.

Received December 6, 1961. P.S.E.B.M., 1962, v109.

Crystals and Concretions in the Vaginae of Persistent-Estrous Mice.* (27288)

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Transplantation of the testis of newborn male rats into littermate females induces persistent estrus by the time of puberty(1). Later investigators have found that androgen (2,3,4) or estrogen(5,6,7,8,9) treatment induces persistent estrus in rats if injections are started shortly after birth. The exogenous hormone presumably causes an irreversible disturbance of pituitary gonadotropin secretion. Induction of persistent estrus by sex steroid injections has not been reported in other mammals, although Perrotta has accomplished this in mice (personal comm.). During our successful attempts to accomplish this in various strains of mice, peculiar vaginal concretions were encountered, which are reported herein.

Materials and methods. 40 A/Crgl, 16 BALB/cCrgl, 15 C3H/Crgl, 16 RIII/Crgl and 24 C57BL/Crgl female mice were injected with 5 μg estradiol-17β in 0.02 ml aqueous suspension for 5 days starting within 24 hours after birth. 20 A, 5 BALB/c, 6 C3H, 4 RIII and 5 C57BL mice served as controls, being injected with 0.02 ml of 0.5% aqueous phenol (the vehicle for the estradiol). The mice were weaned at about 30 days of age. Vaginal smears were recorded daily for various periods of time.

Results. In all estrogen-injected mice, the vagina opened at 5-7 days of age, and clitoridal hypospadias occurred as in the female rat treated with estrogen in prenatal or early postnatal life(10,11,12). Persistent estrus began at 15 to 40 days of age in almost all mice; occasionally, the estrus was interrupted by a diestrous period in about 30% of the mice, not extending for more than 12 days. All control mice generally had normal estrous cycles; however, slightly prolonged estrus or diestrus occasionally occurred in all mice. The controls never showed uninterrupted cornified smears for longer than 9 days. Abnormal estrous cycles and interruption of persistent estrus appear to be more frequent in mice than in rats.

From about 50 days of age, prismatic crystals were noted in vaginal smears of the persistent-estrous mice of every strain (Fig. 1). These crystals resemble the so-called triple phosphates of human urinary sediments. In C57BL mice, numerous crystals were found regularly; about 50% of the daily vaginal smears taken showed crystals (over a 24-day period). About 10% of the vaginal smears from BALB/c mice and about 20% of those from A and RIII mice showed crystals. Crystals were found only rarely in C3H mice; only 2% of the vaginal smears had crystals. No crystals appeared in the control mice.

Large white concretions or clumps of concretions were found in the vaginal lumens of 21 of 40 persistent-estrous A mice at 6-7 months of age (Fig. 2); stones appeared later in an additional 9 mice at about 9 months of age. Many crystals and concretion frag-

* Aided by research grants from Nat. Cancer Inst., U.S.P.H.S. and from Nat. Science Foundation. We are indebted to Dr. R. R. McCormick of Schering Corp. for estradiol.

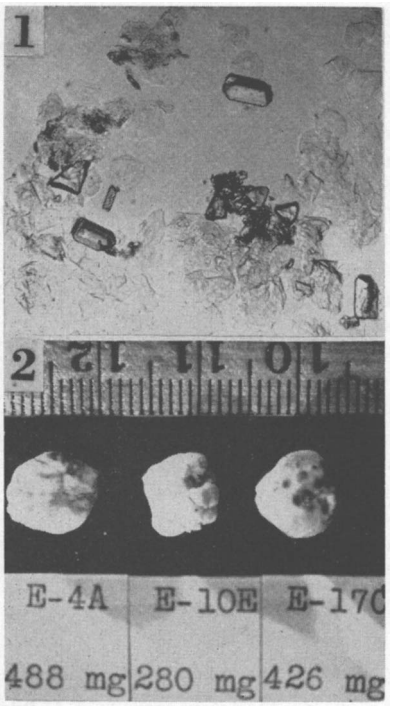

FIG. 1. Crystals and cornified cells in vaginal smear from C57BL/Crgl mouse after about one month of persistent estrus. ×60.

FIG. 2. Concretions removed surgically from vaginae of A/Crgl mice after about 6 mo of persistent estrus.

ments occurred in the vaginal smears of mice bearing the concretions. Eight mice, including 5 with concretions, were killed at about 7 months of age for histologic examination of ovary, uterus and vagina. The ovaries consisted of normal, atretic and cystic follicles, and of hypertrophied interstitial tissue, but completely lacked luteal tissue. Three animals showed fluid-filled oviducts, and 3 showed enlarged or distended uteri. Although 5 mice had thread-like uteri, the uterine epithelium of all mice showed estrogenic stimulation. The vaginal epithelium was highly stimulated and consisted of many epithelial cell layers and a thick stratum corneum. Parakeratotic and inflammatory reactions were evident, presumably in response to the presence of the large concretions. The uterine cervix was also cornified. Vaginal concretions located by palpation were removed surgically from 30 persistent-estrous A mice at 7-10 months of age. The concretions weighed from 52 to 488 mg. About 2 months after the concretions were removed, new palpable concretions appeared in 8 mice and were removed about 3 weeks later. The weight of the second generation of "stones" ranged from 52 to 170 mg. Concretions have also been found in BALB/c and C57BL mice, but less frequently than in A mice at 8 months of age. In other strains concretions have not yet been found (at 6-8 months of age).

Commercial chemical analysis of the concretions indicates that they are composed of about 45% organic matter and about 50% calcium phosphate. Other tests revealed the presence of triphosphate and of acid sodium urate. Sodium phosphate was present after the concretions had been ashed. No compounds of magnesium or ammonium were detected.

Discussion. In various strains of female mice treated with estrogen for long periods, the occurrence of crystals or concretions in the vaginal lumen has never been reported (13). In the persistent-estrous rat, again no crystalline materials were seen in the vagina, even after as long as 605 days(14). Clitoridal hypospadias is produced in neonatal rats by estrogen administration to the antepartum or lactating mother(10,11), or to the newborn female(12). The clitoris is widely cleft, and the urethra opens immediately in front of the vaginal orifice instead of at the apex of the clitoris. Hypospadias in the persistent-estrous mouse is of the same character. One of the sources of material for formation of crystals and concretions may be from the flow of urine into the vaginal lumen. Another possible source is the secretions of the oviduct or uterus, with or without the contributory action of the bacterial flora which proliferate in the estrous vagina(15).

The vaginal crystals resemble those of triple phosphates, which are different from the crystals of calcium phosphate or acid sodium urate. The identity of these crystals is unknown, but they could be ammonio-magnesium phosphate (the triple phosphate of human urinary sediments) or calcium-sodium phosphate, for example. However, the concretions contain no ammonio-magnesium phosphate; accordingly, a relation of the

crystals to concretion formation is suggestive, but not established. Both entities could be independent consequences of the same phenomenon.

Summary. Crystals and concretions were found in the vaginae of A/Crgl, BALB/cCrgl and C57BL/Crgl mice with persistent estrus produced by injection of estradiol-17β for 5 days starting from day of birth. The concretions appeared at 6-7 months of age. Crystals have also been encountered in vaginal smears of C3H/Crgl and RIII/Crgl mice, which have been in persistent estrus for 2 months. The crystals resemble those of triple phosphates; the concretions consist largely of organic matter (including urate) and calcium phosphate.

1. Pfeiffer, C. A., *Am. J. Anat.*, 1936, v58, 195.
2. Selye, H., *Endocrinology*, 1940, v27, 657.
3. Bradbury, J. T., *ibid.*, 1941, v28, 101.
4. Takasugi, N., *Annot. Zool. Japon.*, 1952, v25, 120.
5. Greene, R. R., Burrill, M. W., *Proc. Am. Physiol. Soc.*, 1941, 114.
6. Turner, C. D., *ibid.*, 1941, 283.
7. Wilson, J. G., *Anat. Rec.*, 1943, v86, 341.
8. Hale, H. B., *Endocrinology*, 1944, v35, 499.
9. Takasugi, N., *J. Fac. Sci., Univ. Tokyo*, 1954, Sec. IV, v7, 299.
10. Hain, A. M., *Quart. J. Exp. Physiol.*, 1935a, v25, 131.
11. ———, *ibid.*, 1935b, v25, 303.
12. Greene, R. R., PROC. SOC. EXP. BIOL. AND MED., 1937, v36, 503.
13. Gardner, W. U., *Advances in Cancer Research*, 1953, v1, 173.
14. Takasugi, N., *Annot. Zool. Japon.*, 1958, v31, 74.
15. Everett, J. W., *Endocrinology*, 1939, v25, 123.

Received December 7, 1961. P.S.E.B.M., 1962, v109.

Effect of Selenium on Methionine Formation *in vivo* and *in vitro*.* (27289)

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Hofmeister(1) suggested that salts of selenium and tellurium form methyl compounds in the animal body. He did not mention either the possible methyl source or compounds which serve as methyl donors. du Vigneaud and his school(2) provided the first experimental evidence that transmethylation can occur *in vivo*. They demonstrated that choline, betaine and methionine served as methyl donors. Borsook and Dubnoff(3) have established that choline and betaine can serve as methyl sources in the synthesis of methionine from homocystine, homocysteine and homocysteinethiolactone in tissue homogenates.

Considerable presumptive evidence has been presented in recent years, by use of radioactive selenium, of the biological synthesis of selenium analogues of cysteine(4) methionine and cystine(5,6,7).

* This investigation was aided by a grant from AEC.

† Approved for publication by Director of Experiment Station. Research Publication No. 182.

Rosenfeld and Beath(8) reported that in chronic selenosis total nitrogen and sulfur content of all tissues decreased but significant decrease occurred in the liver.

There is no available information on the effect of chronic selenosis on protein metabolism. This report deals with the effect of chronic selenosis on methionine and cystine content of liver and also the effect of inorganic selenium compounds on methionine formation by transmethylation *in vitro*.

Experimental. Adult Sprague-Dawley rats were used in all studies reported here. A group of 10 rats received daily intraperitoneal injection of 2 mg of selenium as sodium selenate. The animals developed chronic selenosis in 4 to 5 weeks. Toxic symptoms were indicated by considerable loss of weight, emaciation or abdominal ascites, anorexia and terminal aphagia. Animals were sacrificed 2 days after development of aphagia. Liver damage was similar to that reported by Rosenfeld and Beath(9). Since emaciation, anorexia and aphagia may influence protein