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Occurrence of Urinary Calculi in Inbred Strain (C3H) of Mice Treated with Estrogen.

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We have observed in male mice of the C3H* strain many of the pathologic changes in the genitourinary tract reported by previous investigators[1, 2, 3] to follow the injection of large doses of estrogen. We have also observed the occurrence of urinary calculi.

One hundred fifty-one male animals were injected subcutaneously at the age of 2 weeks with Progynon-B (estradiol benzoate in a solution of sesame oil). Twenty-three animals were given 2 doses each of 1500 rat units on alternate days. One hundred fourteen were injected with 100 rat units at weekly intervals for 4, 8, 12, 16, and 20 weeks. In both groups treatment was terminated at the end of these intervals. In 14 other mice similar weekly injections were continued for periods varying from 21 to 35 weeks. Sixty-eight female and 28 male C3H mice were used for controls. The diet consisted of fresh Purina dog chow, with lettuce approximately once a week. Water was always available through a drinking tube.

In 41 experimental animals which died or were sacrificed the presence of calculi was determined by dissection of the urinary tract and roentgen-ray study of the dissected specimens. All the calculi proved radio-opaque. Since an accurate antemortem diagnosis of stones had frequently been made by palpation of the bladder through the abdominal wall, a census was taken of the entire colony, and roentgenograms were made of all mice in which stones could be palpated.

Calculi occurred in 4 (4.1%) of the 96 control mice, and in 50 (33.1%) of the 151 injected mice. All animals under 5 months of age were discarded in our additional calculations for 3 reasons: (1) Only 5 of the 55 injected mice less than 5 months old developed calculi. (2) The incidence of calculi in the injected animals rose from 14.3% in the fifth to 66.6% in the sixth month. (3) None of

* The parent animals were obtained from the Roscoe B. Jackson Memorial Laboratory at Bar Harbor, Maine.

1 Lacassagne, A., *Compt. rend. Soc. de biol.*, 1933, **113**, 590.

2 Burrows, H., and Kennaway, N. M., *Am. J. Cancer*, 1934, **20**, 48.

3 Rusch, H., *Endocrinology*, 1937, **21**, 511.

the control animals under 5 months of age developed calculi. Of the 20 male and 43 female controls in this selected age group, 4 (6.3%), all females, developed stones. Of the 96 injected males in this age group, however, 45 (46.8%) developed calculi.

The incidence of stones varied according to the dose of estrogen administered (Table I). The mice injected with 100 rat units for more than 20 weeks, and those given a total of 3,000 rat units in 2 injections on alternate days, showed a relatively low and almost equal incidence of stones. Mice which had had 9 to 20 weekly injections of 100 rat units showed the highest incidence.

TABLE I.

No. weekly doses 100 rat units estrogen	4	5-8	9-12	13-16	17-20	Above 20	3000 in 2 doses
No. animals with stones	3	7	7	9	11	3	5
% animals with stones	23	58	78	81	73	23	22

The data now available would seem to indicate that stones appear at an earlier age in injected than in control animals. The average age of control animals above 5 months of age without stones was 11 months, 7 days, and of the 4 female controls with stones 11 months, 17 days. The average age of the injected male mice without stones, however, was 8 months, 25 days, and of the 45 with stones 8 months.

The location and appearance of the calculi differed in the injected and the control mice. In the latter urinary stones were found only in the bladder. In the injected mice most of the stones were in the bladder, but occasionally they appeared in the urethra, ureters, and kidneys.

In the injected mice all the calculi were white, chalky in appearance, moderately soft, and sometimes laminated. Usually from 3 to 10 were present in the bladder. The individual stones varied in size from tiny, gravel-like fragments to concretions 0.5 cm in diameter. Often several calculi were cemented together with a soft, chalky material or a tenacious mucoid substance, to form a cast of the entire bladder. In control animals the stones were hard, smooth, discrete, and uniformly 2 to 3 mm in diameter, and not more than 4 were present in any instance.

Summary. Urinary calculi occurred in 33.1% of the 151 strain C3H male mice treated with estogren, and in 4.1% of 96 controls. The highest incidence was noted in mice 5 months of age or older, which had received from 9 to 20 weekly injections of 100 rat units. The calculi appeared at an earlier age in the treated animals.