rhagic hepatic damage and other lesions. Rats of both sexes on still more deficient diets were as resistant as normal rats. Testosterone propionate increased the toxicity of monocrotaline for both sexes on the more deficient diet. These experiments suggest that the greater susceptibility of male rats to monocrotaline may be due to the metabolic effects of male sex hormones.

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## Total 17-Ketosteroid Excretion in Normal and Ovariectomized Rabbits.*

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The excretion levels of the 17-ketosteroids in urine have been studied extensively in humans. Comparisons of the levels of normal and castrate females vary; some workers have reported no significant difference after ovariectomy,[1,2] while others reported below normal levels, but with some overlapping.[3] Friedgood[4] observed a drop following castration, then a rise to normal. In a study on 2 Rhesus monkeys, Dorfman[5] reported a marked rise immediately after gonadectomy, returning to normal by the 22nd day in 1 animal while the other showed only a slight decrease during the first 5 days.

Because the environmental conditions of experimental animals are more easily controlled than those of human subjects, it was considered that the animal affords better opportunity for study of excretion levels under experimental conditions. This report deals with the normal and castrate levels of the total 17-ketosteroids in female rabbit urine.

*Materials and Method.* All animals were of the New Zealand strain, varying in age between 5 months and 2½ years. They were fed complete rabbit pellets with the exception of one change which is discussed later. The animals were housed in separate cages over screened trays which connected collecting flasks. When bilaterally ovariectomized, the animals were allowed at least a 17- to 21-day recovery period before collections were begun. Two animals had been ovariectomized one year previously.

The urine samples were collected for 48-hour periods over 5 ml hydrochloric acid, and were stored at 5°C until used. They were extracted according to the method of Pincus,[6] using a volume of 250 or 300 ml of urine. The crude neutral fractions were treated with Girard "T" in the cold.[6] Colorimetric assay was carried out in absolute alcohol[7] using a Beckman spectrophotometer, with a dehydroisoandrosterone standard.‡ The reaction color obtained with rabbit urine extracts is similar

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1 Fraser, R. W., Forbes, A. P., Albright, F., Sulkowitch, H., and Reifenstein, E. C., *J. Clin. Endocrin.*, 1941, **1**, 234.

2 Hamblen, E. C., Ross, R. A., Cuyler, W. K., Baptist, M., and Ashley, E., *Endocrin.*, 1939, **25**, 491.

3 Callow, N. H., Callow, R. K., and Emmens, C. W., *J. Endocrin.*, 1940, **2**, 88.

4 Friedgood, M., *A.A.A.S. Symposium: The Chemistry and Physiology of Hormones*, 1944, pp. 195-202.

5 Dorfman, R. I., Horwitt, B. N., Shipley, R. A., Fish, W. R., and Abbott, W. E., *Endocrin.*, 1947, **41**, 470.

6 Pincus, G., *J. Clin. Endocrin.*, 1945, **5**, 291.

7 Talbot, N. B., Butler, A. M., and MacLachlan, E., *J. Biol. Chem.*, 1940, **132**, 595.

‡ Crystalline dehydroisoandrosterone was generously supplied by Ciba Pharmaceutical Products, Inc., and by Schering Corporation.

TABLE I.
17-Ketosteroid Excretion and Urine Volumes in Normal Female Rabbits.

| Group | Animal | No. of runs | Avg 17-ks. mg/48 hr | Avg urine vol. ml/48 hr |
|---|---|---|---|---|
| 1 | A | 13 | 1.50 | 544 |
| | B | 8 | 1.36 | 275 |
| | C | 11 | 1.50 | 358 |
| | D | 6 | 1.38 | 344 |
| | Total | 38 | 1.45 | 400 |
| | | | S.D. = 0.07 | |
| 2 | I | 9 | 2.56 | 441 |
| | J | 6 | 4.22 | 592 |
| | K | 7 | 3.53 | 488 |
| | L | 5 | 5.79 | 555 |
| | M | 12 | 4.45 | 593 |
| | N | 12 | 3.72 | 572 |
| | O | 6 | 3.33 | 336 |
| | P | 6 | 4.00 | 444 |
| | Total | 63 | 3.88 | 515 |
| | | | S.D. = 0.89 | |

TABLE II.
17-Ketosteroid Excretion and Urine Volumes in Castrate Female Rabbits.

| Group | Animal | No. of runs | Avg 17-ks. mg/48 hr | Avg urine vol. ml/48 hr |
|---|---|---|---|---|
| 1 | E | 7 | 1.34 | 266 |
| | F | 6 | 1.29 | 312 |
| | G | 5 | 1.89 | 318 |
| | H | 6 | 1.84 | 456 |
| | Total | 24 | 1.56 | 335 |
| | | | S.D = 0.27 | |
| 2 | I | 6 | 4.44 | 275 |
| | J | 9 | 4.95 | 449 |
| | K | 7 | 4.61 | 374 |
| | A | 6 | 2.77 | 314 |
| | Total | 28 | 4.29 | 365 |
| | | | S.D. = 0.85 | |

to that developed with human urine extracts. All blanks and extracts were assayed in triplicate. A total of 101 normal and 52 castrate urine samples were run in this manner.

*Reagents.* Ether for extractions, U.S.P. grade, was treated with ferrous sulfate and thoroughly washed with distilled water. Other reagents were of C.P. grade. The absolute ethanol was further dried and purified. *m*-dinitrobenzene was recrystallized until nearly colorless.

*Results and discussion.* The results are shown in Tables I and II. The amount of the 17-ketosteroids excreted by 4 normal intact rabbits is, for Group 1, 1.45 ± 0.07 mg, for 4 castrate animals the corresponding level is 1.56 ± 0.27. The normal for the 8 animals of Group 2 is 3.88 ± 0.89 mg, with the castrate level being 4.29 ± 0.85 for 4 animals. Both normal and castrate levels are shown for animals J, K, and I.

Although the average difference in 17-ketosteroid excretion appears slightly higher in castrate than in normal rabbits, a statistical analysis shows that there is no significant difference between them.§ These data are in

§ $\dfrac{M_{normal} - M_{cast.}}{S.E.\ M_n - M_c}$ / 3 (Group 1 = 2.1) (Group 2 = 2.0)

agreement with those presented by Fraser *et al.*,[1] Hamblen *et al.*,[2] and Callow *et al.*,[3] and contrary to the findings of Friedgood.[4]

It will be noted that in both normal and castrate animals (Tables I and II) there are two groups of animals, each showing different values. The only reason for division into 2 groups was: (a) in both Tables I and II the animals of Group 1 were older than those in Group 2 (1 to 2½ years as compared with 5 to 6 months); (b) the complete rabbit pellets fed were changed abruptly and unintentionally so that all animals in Group 1 received one brand of pellets, while those of Group 2 received a different brand (this change was instituted by the animal caretakers). It was for this reason that the animals were considered separately, and not because of their ketosteroid values.

The average urine volumes are also shown in Tables I and II. It was found that there is no statistical correlation between urine volume and 17-ketosteroids excretion in normal or castrate animals. (Correlation factor $r = -0.119 \pm 0.095$ for normals, $-0.118 \pm 0.136$ for castrates, showing an insignificant negative correlation). This is in agreement with work done by Kimmeldorf on male rabbits,[8] as well as by Bachman *et al.*,[9] and Pincus[10] on humans.

*Summary.* 1. Total 17-ketosteroid urinary levels have been determined for normal and ovariectomized rabbits. There is no statistical difference between them. 2. The ratio between urine volume and 17-ketosteroid excretion has been examined statistically and no correlation found.

[8] Kimmeldorf, D., (unpublished data obtained in these laboratories).

[9] Bachman, C., Leekley, D., and Winter, B., *J. Clin. Endocrin.*, 1941, **1**, 142.

[10] Pincus, G., *J. Clin. Endocrin.*, 1943, **3**, 195.

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## Effect of Orally Administered Sodium Estrone Sulfate on Total 17-Ketosteroid Excretion in Female Rabbits.*

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The effects on the total 17-ketosteroid excretion after estrogen administration have not been studied extensively. Hamblen and his co-workers[1] studied the change in 17-ketosteroid excretion titers after estrogen administration to women in the climacterium, those complaining of hypoovarianism or functional uterine bleeding.

This study was undertaken to determine the effect, if any, of the oral administration of sodium estrone sulfate (Premarin‡) on the total 17-ketosteroid excretion in normal and ovariectomized rabbits.

*Materials and methods.* Six female rabbits of the New Zealand strain, 4 normal and 2 castrate, were used for this experiment. The age of the animals varied from 7 months to 2-2½ years. Animals V and VI were bilaterally gonadectomized 2 months before being used in this experiment. All animals were kept in separate cages and fed complete rabbit pellets.

The urine was collected for 48-hour periods over 5 ml HCl. It was extracted and purified with Girard "T" according to Pincus,[2] and

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† The authors wish to express their thanks to Mr. Henry Rubin for his valuable technical assistance.

[1] Hamblen, E. C., Pattee, C. J., and Cuyler, W. K., *Endocrin.*, 1940, **27**, 734.

‡ Generously supplied by Ayerst, McKenna and Harrison, Ltd.

[2] Pincus, G., *J. Clin. Endocrin.*, 1945, **5**, 291.