increased the proteinuria, while a diet with only 5% casein diminished it markedly.

Since the proteinuria in mice is associated with sex, the effect of castrating young male mice was studied. The degree of proteinuria remained unchanged for many weeks following the operation, but was greatly decreased after about 3 months. Addis[9] has reported a similar result for albuminuria of male rats. Bell[10] noticed the appearance of albuminuria as young male rats reached maturity. Our rats show no excretion of protein, and we have found very little mention of any marked proteinuria of these animals.

In connection with the apparent sex-linked character of the proteinuria of mice, the recent report of Crabtree[11] is of great interest. She observed differences in structure of Bowman's capsule between most male and female mice. She cites the experiments of Selye,[12] who noticed that treatment of female mice with testosterone propionate may change the parietal lamina of Bowman's capsule.

Summary. A protein with chemical characteristics of a nucleoprotein was found to be excreted in large amounts by male mice of various ages and strains. Female mice excrete much less protein, frequently none at all. A table is appended.

For the castration of animals and the examination of sections, I am much indebted to Dr. William Cramer.

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Prolongation of Estrus by Injection of Suspensions of Estrogen Crystals.

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The influence of the method of administration upon the duration of the effect of estrogenic compounds has early been recognized

9 Addis, T., *Proc. California Acad. Med.*, 1931-32, 38, and personal communications.

10 Bell, M. E., *J. Physiol.* (Brit.), 1933, **79**, 191.

11 Crabtree, C., *Science*, 1940, **91**, 299.

12 Selye, H., *J. Urol.*, 1939, **42**, 637.

and described by several authors.[1] D'Amour and Gustavson[2] showed that 3.8 γ estrone in aqueous solution can produce estrus in rats when the dose is given in a single injection, while only 0.74 γ is necessary if multiple injections are administered. A solution in oil, however, does not show significant differences if administered in one or fractional doses. This is in agreement with the findings of Behrens and collaborators[3] that the spreading of the M.E.D. of an aqueous solution of estrone resulted in prolongation of estrus. Parkes[4] showed that even massive doses of estrone produced only a transitory feminization of the plumage of Brown Leghorn capons. Similarly, Miescher and collaborators[5] found that estrus in rats after subcutaneous injection of as much as 50 γ estrone in oil lasted not more than 5 days. Deansly and Parkes[6] implanted crystals of 2.0 to 3.2 mg estrone intramuscularly in Brown Leghorn capons and found that the feminization of the plumage lasted for approximately 3 months. Recently, it has been shown that the implantation of estrogen crystals or pellets is highly efficient in therapeutics.

Experimental. Obviously, the implantation of estrogen crystals is not a simple procedure. The administration of a suspension of estrogens, as for instance, 5 mg estrone in 1 cc of an aqueous solution of 5.2% gum acacia, is a practical modification of the implantation method. The vehicle is promptly resorbed and leaves the finely divided crystals in the tissues. The crystals are small enough to pass through a 26 gauge hypodermic needle. The suspended estrone settles very slowly and a suspension can be obtained by shaking the mixture before injection. A suspension of 5 mg estrone in 1 cc sesame oil was also prepared. Due to the solubility of the estrone in the oil, approximately 1 mg is present in solution and the rest in suspension. Furthermore, an aqueous suspension was made from estradiol, containing 5 mg in 1 cc of 5.3% gum acacia solution. Microscopical examination of the suspensions showed a very satisfactory uniformity regarding the size of the crystals.

Castrated female rats and mice, as they are employed for assay work of estrogens, were used. Rats received one single injection

[1] Allen, E., Hisaw, F. L., and Gardener, W. W., *Sex and Internal Secretion*, E. Allen, Baltimore, 1939.

[2] D'Amour, F. E., and Gustavson, R. G., *J. Pharm. and Exp. Therap.*, 1936, **57**, 472.

[3] Behrens, B., Heubner, W., Koll, W., and Kulz, F., *Arch. Exp. Path. und Pharm.*, 1937, **186**, 121.

[4] Parkes, A. S., *Biochem. J.*, 1937, **31**, 579.

[5] Miescher, K., Scholz, C., and Tscopp, E., *Biochem. J.*, 1938, **32**, 141.

[6] Deansly, R., and Parkes, A. E., *Proc. Royal Soc., London*, 1937, **124**, 279.

TABLE I.
Duration of Estrus in Rats and Mice Following Injection of Estrogens.

Series	No. of animals	Dose, mg	Vol., cc	Duration of estrus (days) Range	Avg	Drug used
				Rats.		
I	9	.5	.1	5-20	15.5	Estrone, aqueous suspension
II	10	.5	.1	6-24	15.0	" " "
III	10	.5	.1	11-39	23.1	" " "
IV	9	.5	.1	13-41	23.5	" " "
V	10	.5	.1	9-19	14.2	Estradiol, " "
VI	9	.5	.1	5-20	9.6	Estrone, oil suspension
VII	9	.5	.4	2.0	2.0	" solution in oil
				Mice.		
I	10	.25	.05	10-26	18.0	Estrone, aqueous suspension
II	10	.15	.10	13-40	20.8	" " "
III	10	.15	.10	9-21	15.3	" " "
IV	10	.15	.10	7-20	10.6	Estradiol " "
V	6	.25	.20	4-10	6.0	Estrone, solution in oil

of 0.1 cc subcutaneously, amounting to 0.5 mg of the estrogens in the form of suspensions as illustrated in Table I. As a control, 0.5 mg estrone dissolved in 0.4 cc oil was given to Group VII. Ninety percent of the animals in all groups came into estrus within 24 hours, the rest within 48 hours.

Duration of estrus was followed by vaginal smears in each animal and the average for each group was determined. The estrus in the rats injected with a solution of estrone in oil (Group VII) lasted only 2 days, while the effect was very much prolonged in all groups receiving an estrogen in a form of a suspension both in oil and in water. There was a great variation in the duration among the different animals within the groups. However, the averages show a significant prolongation of effects with the aqueous suspension of estrone in the Groups I-IV and with estradiol in Group V.

The estrus obtained with the estrone suspension in oil was definitely shorter in duration than that with estrone in water. Probably the dissolved estrone is removed from the oil in the tissues and the suspended estrone dissolves again in the remaining oil, thus hastening the absorption as compared with the aqueous suspension.

The experiments in mice essentially corroborate the findings in rats. Doses of 0.25 and 0.15 mg were used. While the duration of estrus following the injection of a solution of 0.25 mg of the estrone in oil is longer than that resulting from the injection of 0.5 mg in oil in rats, the aqueous suspension at both dose levels used in mice results in a prolongation of estrus of the same order as observed in rats. The injection of estradiol in an aqueous suspen-

sion produced also some prolongation of the duration of estrus. A number of rats and mice were sacrificed during the later period of the estrus and the uteri examined histologically. They presented hypertrophy of the muscular layer and submucosa. The changes were more pronounced in the rats and in these the effect was more marked in the animals injected with the aqueous suspension than with the suspension in oil.

Inspection of the places of injection when the aqueous suspension was used showed no irritation, nor were deposits visible. In addition to the convenience of administration, the freedom of oil eliminates the possibility of reactions as they occasionally occur in patients sensitive to oil. Clinical investigation in humans, using the same aqueous suspension of estrone, corroborates the results in experimental animals.[7]

Conclusions. The duration of estrus in castrated rats and mice was measured after injection of equal amounts of estrone as an aqueous suspension, as a suspension in oil, and as a solution in oil. The suspensions, particularly the aqueous ones, by far outlasted the effect of the solution. The aqueous suspension of estradiol was also followed by a prolonged stage of estrus. This method offers a convenient way to produce prolonged effects with free estrogens without resorting to surgical implantation of crystals.

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Effect of Vitamin C Deficiency on Action of Different Types of Barbiturates.

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Generally, the liver is assumed to be of importance for the destruction of the so-called short-acting barbiturates, but not for the long-acting group. Recent work by Scheifley and Higgins[1] using partial hepatectomy, and by us[2] employing chemical liver damage, brought the unexpecteed result that the liver does not seem to be equally important for the destruction of pentothal sodium, a widely used ultra-short-acting intravenous anesthetic. Simultaneously, we

[7] Freed, S. C., *J. Clinic. Endocrin.*, in press.

[1] Scheifley, C. H., and Higgins, G. M., *Am. J. Med. Sci.*, 1940, **200**, 264.

[2] Richards, R. K., and Appel, M., *Anesthesia and Analgesia*, 1941, **20**, 66.