167 mg sodium pregnanediol glucuronidate per gallon, an amount well within the range of estimation by the Venning[3] procedure. We therefore applied this technic to urine samples obtained from the bladders of 4 freshly slaughtered bulls and 2 steers, the volumes being 1360, 840, 320, 300, 480, and 1060 cc respectively. Each urine was promptly extracted with butanol, and run through the usual method as for human urine.[3] The extracted urine, the extracted 0.1 N NaOH and washings, were pooled and set aside for hydrolysis.

The steer urine appeared to be greatly dilute compared to that of the bulls.

On evaporation of the second butanol extracts under reduced pressure, white material was noted, but on the usual precipitation from 5 cc water and 95 cc anhydrous acetone no precipitate was obtained except in one case, where on reprecipitation and weighing, 3.7 mg of material melting instantaneously at 167° was found. The acetone solutions were evaporated and those of the 4 bulls pooled. These combined residues (from 2820 cc of bull's urine) weighed 1.2 g and contained an easily sublimed white material, but none of the easily recognizable sodium pregnanediol glucuronidate.

Summary. No sodium pregnanediol glucuronidate was found in urine obtained from the bladders of slaughtered bulls and steers by the method of Venning.[3] The volumes of urine were judged sufficient for demonstration of this substance if it were present in the amount suggested by Marker's results for pregnanediol 3α, 20α from hydrolyzed bull's urine.

13213

Bactericidal Property of Blood Serum of Male Rabbits Treated with Urinary Estrogens.

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There is clinical and experimental evidence of a definite relationship between hormones and immunity to infection. The early work on the effect of the suprarenal glands on resistance to disease was reviewed by Perla and Marmorston.[1, 2] The effect of insulin on

[1] Perla, D., and Marmorston, J., *Arch. Path.*, 1933, **16**, 379.

[2] Perla, D., PROC. SOC. EXP. BIOL. AND MED., 1934-35, **32**, 797.

infection has also attracted much attention.[3] Recently, Cope and Kapnick[4] reported changes in complement-concentration and in response to vaccinial infection following alterations in thyroid, adrenal, and pituitary function in the rabbit and dog.

Pregnant mare's serum has been shown to neutralize *in vitro* the virus of poliomyelitis,[5] and Aycock[6] has found that treatment with estrogenic hormone protected castrated monkeys against the same disease. Sprunt and his co-workers[7] observed the favorable effect of estrogenic and gonadotropic hormones on vaccinial infection in rabbits, and Weinstein[8] has recently demonstrated the prophylactic value of certain hormonal preparations, such as those of the anterior pituitary gland and parathyroid, against anthrax in mice. He failed, however, to find any effect of estrin on this infection. Working on adult rabbits treated with estrogenic hormone, the same author[9] demonstrated an increase in the circulating agglutinin for *E. coli* and hemolysin for sheep cells.

There have also been numerous studies of the relation of sexual maturity to immunological reactions.[10]

The relationship between sex-hormones and immunity to syphilis was suggested by the observation of Frazier, Mu, and Hu[11] in this laboratory. It has been shown that estrogenic hormones extracted from the urine of pregnant women enhance the resistance to syphilitic infection of male rabbits feminized by the action of such substances.[12]

The purpose of the present paper is to report a study of the effect of estrogenic hormone on the natural antibody-level of adult male rabbits as measured by the bactericidal property of their blood serum on *E. typhi.*

Material and Method. A total of 72 adult albino rabbits were used, of which 60 were male and 12 female. Of the 60 male rabbits, 29 were treated 6 times a week with 40 R.U. of estrogen (equivalent approximately to 102 international units of estrone) in olive

[3] Marble, A., White, H. J., and Fernald, A. T., *J. Clin. Invest.*, 1938, **17**, 423.

[4] Cope, O., and Kapnick, I., *Endocrinology*, 1940, **27**, 533.

[5] Jungeblat, C. W., and Engle, E. T., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 879.

[6] Aycock, W. L., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 573.

[7] Sprunt, D. H., McDearman, S., and Raper, J., *J. Exp. Med.*, 1938, **67**, 159.

[8] Weinstein, L., *Yale J. Biol. and Med.*, 1938-39, **11**, 369.

[9] Weinstein, L., *Yale J. Biol. and Med.*, 1938-39, **11**, 169.

[10] Baumgartner, L., *Yale J. Biol. and Med.*, 1933-34, **6**, 403.

[11] Frazier, C. N., Mu, J. W., and Hu, C. K., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 65.

[12] Frazier, C. N., and Hu, C. K., *Endocrinology*, 1941, **28**, 283.

oil for 300 days or more. This extract was prepared in our laboratory according to the technic previously described.[12] Fifteen of the rabbits were treated with the same amount of olive oil as control. The other 16, together with the female rabbits, were not treated. The male rabbits were not castrated.

Blood was withdrawn from the ear under aseptic conditions. The serum was separated and used without dilution or heating. The organisms used, *E. typhi,* were grown on meat-infusion agar for 18 hours at 37°C. At the end of that time they were washed off with saline, making an even suspension of 320 million organisms per cc, from which serial dilutions were made; 0.1 cc of the serum was added to an equal amount of the different dilutions of the bacterial suspension. A serum-control without organisms was included in each sample. The tubes were well shaken and incubated at 37°C for 2 hours. At the end of this time, 3 to 5 cc of blood-digest broth with 1% dextrose and litmus indicator were added to each tube, and the mixtures were again incubated at 37°C for 48 hours. The final result was observed at the end of the second incubation. The lowest dilution of the suspension in which there was no evidence of growth was taken as the endpoint.

One control determination was made on each animal before treatment, and a second determination after 6 days of treatment. After these two determinations, bleeding was done at different intervals varying from 25 to 300 days or more after the beginning of treatment. Some of the animals were tested more than once.

Results. The results are recorded in Table I. The figures represent the number of organisms destroyed and are based on the mean values of the total number of animals in each group, indicated by *N,* together with their standard deviations. The significance of the differences between the various groups was estimated by the statistical *t*. *P* represents the probability of the difference being due to chance.

Discussion. From the table, one can see readily that there was a marked increase of the bactericidal power of the blood serum after one week of treatment with estrogen. As the treatment was prolonged, there was an increase of bactericidal power as compared with the status of the normal male rabbits before treatment. The reason for the inclusion of the results on the group treated for 25 days with those of 300 days was that the mean value of the 12 determinations in the former group failed to show any significant variation from the figures obtained in the latter group. That the increase in bactericidal power was not due to the olive oil in which

TABLE I.
Effect of Estrogenic Treatment on Bactericidal Power of Blood Serum of Rabbits in Terms of Millions of Organisms Killed.

	Groups of comparison				
	Normal male	Treated male	Difference	*t*	*P*
6 days of estrogenic treatment	1.287 ± 0.29 *N* = 17	7.529 ± 1.34 *N* = 17	6.242	8.568	0.001
25 to 300 days of estrogenic treatment	0.907 ± 0.13 *N* = 60	1.743 ± 0.29 *N** = 39	0.836	2.970	0.01-0.001
Oil treatment	0.858 ± 0.27 *N* = 15	0.967 ± 0.09 *N* = 15	0.109	0.3445	0.8-0.9
	Normal male	Normal female			
No treatment	0.907 ± 0.13 *N* = 60	0.667 ± 0.17 *N* = 12	0.240	0.7758	0.4-0.5

*The number of tests among 27 animals.

the estrogenic substance was dissolved is shown by the lack of significant difference with results before and after treatment with the oil alone. The bactericidal property of the blood from normal male rabbits did not differ significantly from that of normal female rabbits.

Conclusion. The administration of urinary estrogens increased the bactericidal property of the blood serum of adult male rabbits against *E. typhi.* This increase was greater shortly after the beginning of treatment. With prolonged treatment, the increase was less apparent but still significant up to the end of the experiment, that is to say, after 300 days of treatment. Whether this was a direct response to the estrogen administered or secondary to other changes in the body induced by hormonal action needs further investigation.

13214

In vitro Effect of Sulfanilamide, Sulfapyridine and Sulfathiazole on *Corynebacterium diphtheriae.*

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The purpose of the present investigation is to determine the effect *in vitro* of sulfanilamide, sulfapyridine and sulfathiazole on *C. diphtheriae.* Observations are also made as to how long it takes for effective action, and on the effect of sulfanilamide, sulfapyridine and sulfathiazole on various serological types of Mitis