

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
No treatment		Normal male 0.907 ± 0.13 N = 60	Normal female 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.

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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

organisms. Nitti, Phillips and Bovet reported that sulfanilamide retards the development of diphtheria bacilli in culture.¹ In the same year, Rosa treated diphtheria carriers by applying the drug locally but failed in this attempt.²

Methods and Materials. One to 1,000 dilutions of sulfanilamide and sulfapyridine were prepared from a 1% sulfanilamide solution and Soludagenan (1 g/3 cc) respectively, and standardized by means of the Klett-Summerson photo-electric colorimeter.* One to 1,000 dilution of sulfathiazole was prepared by heating aqueous solution containing a definite weight of the chemical. Final dilutions of 1:10,000 or 10 mg % were prepared by adding 0.2 cc of stock solution to 1.8 cc of liver digest broth, pH 7.6, containing 5% horse serum. All strains of *C. diphtheriae* were local strains isolated from the throats or noses of patients or carriers and were virulent to Chinese hamsters. One-tenth cc of 1:1,000 dilution of a 24-hour broth culture was used as the inoculum of the first 2 experiments, and a loopful of undiluted 24-hour cultures used in the third one. Survival of organisms was determined by growth on blood agar plates.

Experiment 1. The results of the experiment are summarized in Table I. It represents one of the 3 identical experiments. Under similar conditions, sulfathiazole seems to have exerted a very definite bactericidal action on representative strains of all the 3 cultural types of *C. diphtheriae*, while the other 2 were less effective on one Mitis strain than on strains of the other cultural types.

Experiment 2. In this experiment, frequent subcultures were made at definite intervals on tubes containing given amounts of organisms and 1:10,000 dilutions of sulfathiazole. It was found that the bactericidal activity of the chemical on all the same 3

TABLE I.
Number of *C. diphtheriae* in Broth Containing 10 mg % Sulfanilamide, Sulfapyridine and Sulfathiazole Before and After 48 Hours of Incubation.

Chemical	Mitis (3458)		Intermedius (Park 8)		Gravis (776)	
	Before	After	Before	After	Before	After
Sulfanilamide	10 ⁵	29x10 ⁹	5x10 ⁵	100	6x10 ⁵	160
Sulfapyridine	10 ⁵	26x10 ⁷	5x10 ⁵	1080	6x10 ⁵	0
Sulfathiazole	10 ⁵	0	5x10 ⁵	0	6x10 ⁵	0
Control	10 ⁵	18x10 ⁹	5x10 ⁵	55x10 ⁹	6x10 ⁵	82x10 ⁹

¹ Nitti, F., Phillips, M., and Bovet, D., *Compt. rend. Soc. de biol.*, 1939, **131**, 70.

² Rosa, A., *Bull. Soc. Ital. Biol. sper.*, 1939, **14**, 630.

* All determinations were kindly performed by Dr. S. Y. Pan.

strains did not start until at least 4-8 hours of incubation have elapsed.

Experiment 3. Forty-three strains of *C. diphtheriae* belonging to 6 different serological types³ were tested for their susceptibility to 1:10,000 dilutions of sulfanilamide, sulfapyridine and sulfathiazole. One loopful of 24-hour cultures was employed as inoculum, which is much larger than those previously used. The results are presented in Table II. It was observed that different strains of the same serological type reacted differently to the various chemicals, and that sulfathiazole is by far the most efficient bactericidal agent of the three. All those strains which were susceptible to sulfanilamide were invariably susceptible to sulfapyridine and sulfathiazole. A few of the strains which were found to be resistant to sulfathiazole, when retested with a smaller inoculum (0.1 cc of 1:1,000 dilution of a 24-hour culture), turned out to be susceptible also to the same chemical, a finding which is in agreement with those reported by others on other bacteria.

Comment. The present experiments have confirmed and extended the observations made by Nitti and his coworkers. Not only were the growth of these organisms retarded, marked bactericidal action of several common sulfanilamide compounds, on *C. diphtheriae* with appropriate inoculum was observed. Of the 3 chemicals tested, sulfathiazole was active on the largest number of strains studied, and sulfanilamide least. That it requires 4-8 hours of contact between the chemicals and the organisms before their bactericidal action can be appreciated might explain the failure in the treatment of diphtheria carriers by local application as reported by Rosa.

TABLE II.
Susceptibility of Various Serological Types of *C. diphtheriae* to 10 mg % of Sulfanilamide, Sulfapyridine and Sulfathiazole.

Serological types	No. of strains tested	No. of strains susceptible to		
		Sulfanilamide	Sulfapyridine	Sulfathiazole
II	2	2	2	2
III	12	6	7	8
IV	2	1	1	2
V	13	1	4	6
VI	1	1	1	1
VIII	13	6	6	9
Total	43	17	21	28

³ Huang, C. H., in press.