cycles were injected with testosterone propionate (925 and 1,225 mg), in order to determine whether ovulation could be inhibited. In one patient, the ovaries, examined on the 34th day of the cycle, showed no evidence of a recent corpus luteum or mature graafian follicle. In the second patient, examination of the ovaries, on the 17th day of the cycle, did not reveal any evidence of ovulation. In the latter case, while ovulation might have occurred after the 17th day, it was deemed unlikely in an individual with a regular 26 to 28 day cycle.

It appears from this study that testosterone propionate, if administered in adequate amounts to the cyclical human female, can inhibit full follicle maturation, ovulation and corpus luteum formation, associated with regressive changes in the endometrium and vaginal mucosa. The question arises as to whether the testosterone propionate acts directly upon the follicular apparatus or indirectly through inhibition of the gonadotropic activity of the pituitary. In view of the fact that testosterone has been shown to suppress the gonadotropic activity of the hypophysis in post-menopausal women[14, 15] and rats,[16-18] it is logical to conclude that the inhibitory effect of testosterone propionate upon the human ovary is mediated through the pituitary.

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Effectiveness of Sulfanilamide upon Anaerobic Hemolytic Streptococci.

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(Introduced by J. A. Kolmer.)

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Previous work in this laboratory[1] has indicated that on primary isolation a significant proportion of hemolytic streptococci are incapable of developing upon the surface of aërobic, infusion blood-

14 Salmon, U. J., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 488.

15 Nathanson, I. T., and Towne, L. E., *Endocrinology*, 1939, **25**, 754.

16 Nelson, W. O., and Gallagher, T. F., *Anat. Rec.*, 1935, **64**, 129.

17 Wolfe, J. M., and Hamilton, J. B., *Endocrinology*, 1937, **21**, 603.

18 Allanson, M., *Proc. Roy. Soc., London*, s.B., 1937, **125**, 196.

1 Spaulding, E. H., and Goode, W., *J. Lab. and Clin. Med.*, 1939, **25**, 305.

agar plates. Although most of these "anaërobic" isolations become quickly adapted to aërobic cultivation (temporarily anaërobic), a small percentage persist as obligate anaërobes. As a result of the clinical observation that several patients infected with obligately anaërobic hemolytic streptococci (Group A) responded poorly to sulfonamide-therapy, two such strains were selected for experimental study. The results are being reported because of the increasing interest in the relationship between anaërobiosis and sulfanilamide activity.[2-5]

Both strains reacted with Group A antiserum, and fermented trehalose but not sorbitol. Strain S, originating from a case of bronchiectasis, was unable to grow on aërobic blood-agar for 18 months. The other (1097) was isolated from a hand lesion. After 14 months it began to develop aërobically. Strain S was characterized by mucoid colonies on blood agar, whereas those of 1097 were of the smooth type. On benzidine blood agar[7] both cultures gave rise to black colonies within two hours after removal from anaërobic jar.

Experimental infection in mice was produced with considerable difficulty since only after prolonged passage did death regularly follow the injection of several million cells. It should be noted that streptococcal strains of low virulence do not usually show marked response to sulfanilamide in mice,[6] presumably because of the large number of organisms in the inoculum. Infection was produced intraäbdominally and the drug administered subcutaneously in 10 mg doses per 25 g body weight. The results are summarized in Table I.

It will be noted that neither treatment-schedule was intensive. Nevertheless, it seems evident that infection induced by one strain (S) was definitely refractory to treatment, whereas infection with the other strain (1097) was moderately susceptible to sulfanilamide therapy. Comparative experiments concerning the effect of fresh preparation of sulfanilamide and samples of the drug oxidized by exposure to air for 30 days yielded similar results.

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2 Fox, C. L., German, B., and Janeway, C. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 184.

3 Warren, J., Street, J. A., and Stokinger, H. E., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 208.

4 Shinn, L. E., Main, E. R., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 640.

5 Broh-Kahn, R. H., *Science*, 1939, **90**, 543.

6 Marshall, E. K., Jr., *Physiol. Rev.*, 1939, **19**, 240.

7 MacLeod, C. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 215.

TABLE I.
Therapeutic Effect of Sulfanilamide in Mice upon Anaërobic Hemolytic Streptococci.

Strain	No. of organisms: No. of mice	No. of organisms: injected (millions)	No. of organisms: Lethal doses	Drug	Death of mice in days: 1	2	3	4-6	7-10	11-25	Survived	Autopsy cultures: Aëro.	Anaëro.
S	45	± 25	5-10	Treated*	34	9	2						
S	20	± 25	5-10	Controls	17	3						—	+
S	18	80	10	Treated†	9	8	1					—	+
S	18	80	10	Controls	18							—	+
S	30	16	5-10	Treated*	25	3	2					—	+
S	5	16	5-10	Controls	5							—	+
1097	23	3.4	5-10	Treated*	1	2	5	3	3	1	8	—	+
1097	13	3.4	5-10	Controls	11	2						—	+
1097	17	88	5-10	Treated†	6	2	2		1		6	?‡	+
1097	7	88	5-10	Controls	7							?‡	+
1097	15	6	10	Treated*	1		3	2	3	1	5	—	+
1097	5	6	10	Controls	4	1						—	+

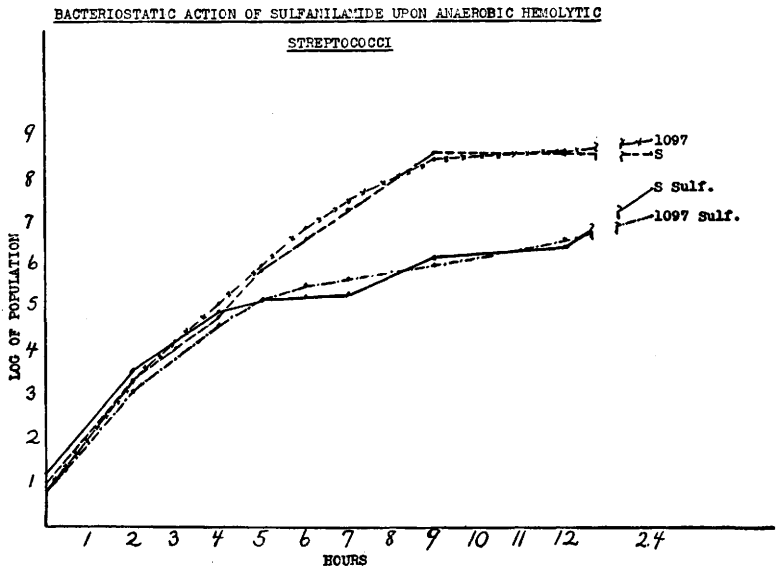
Treatment:
*20 mg/25 g body wt daily for 6 days; 10 mg for 5 days (10 mg doses).
†10 mg/25 g body wt daily for 12 days (10 mg doses).
‡Occasional minute colony; aërobic subculture negative.

The *in vitro* experiments of Fox, *et al.*,[2] and of Warren, *et al.*,[3] suggest that sulfanilamide would be ineffectual against obligately anaërobic strains. The *in vivo* results with strain 1097, however, indicate that anaërobiosis *per se* may not be an important factor in determining susceptibility of hemolytic streptococci to sulfanilamide-therapy.

In vitro bacteriostasis. Since there appeared to exist unequal responses to sulfanilamide between the 2 strains in mice, it was considered possible that *in vitro* tests might bring to light some essential difference between the two organisms. Bacteriostatic tests were performed by adding to 17 cc of peptone-free, 25% serum, infusion broth 2 cc of sulfanilamide to make a final concentration of 1:10,000. The medium was freshly prepared and incubated overnight, anaërobically. One cc of a diluted 15-hour broth culture was added with the introduction of as little oxygen as possible. Platings were made after 2, 4, 5, 6, 7, 9, 12 and 24 hours' incubation anaërobically at 37° C. At the same time the gross turbidity was estimated by barium sulfate standards and the average number of cocci per chain determined microscopically. The graphic results appear in Figure I.

Unlike the results of the mouse experiments both strains were inhibited by sulfanilamide *in vitro*. Since it is well known that in

Figure I



broth containing this drug streptococci produce long chains, the average number of cocci per chain was determined at the time each plating was made. The error due to interpreting the failure of the organisms to divide as true bacteriostasis would have been slight, however, in this instance. There were only 3 times as many elements in the drug-broth as in the control tubes, while bacteriostasis as determined by platings was in the order of a two-hundred fold difference at 9 hours.

Phagocytic Experiments. The discrepancy between the drug-resistance of strain S *in vivo* and its susceptibility *in vitro* was attributed to the enormous difference in the number of bacteria used in the two types of experiments. In mice the number was very large; in the test tube it was small. Lockwood[10] has demonstrated the antibacteriostatic effect of large inocula *in vitro*. Nevertheless, it occurred to us that this strain might show marked resistance to phagocytosis in the presence of sulfanilamide. Therefore, a series of 3 *in vitro* phagocytic experiments was carried out. Using one set of cultures throughout, the test conditions were varied so as to include the use of organisms previously exposed to 1:10,000 sulfanilamide and others not subjected to the drug. The tests were incubated in the water bath at 37° C. with constant rotation. Smears were made after 30 minutes and one hour. Phagocytic activity was estimated by counting 200 leucocytes stained by Wright's method and by determining microscopically the average number of cocci per phagocyte.

A detailed description of the technique is not warranted since it was found that neither strain was markedly influenced by the presence of the drug. Strain 1097 was, perhaps, more readily phagocytized, with or without the drug, than was the S strain.

Further attempts to differentiate strains S and 1097. Because the results in mice had indicated that the strains behaved differently toward sulfanilamide, a series of fermentative tests and dehydrogenase determinations was conducted. The fermentative capacities were similar, however, and dehydrogenase studies, patterned after MacLeod[7] were essentially negative.

Adaptation to aërobic cultivation. After 14 and 18 months respectively strains S and 1097 both developed spontaneously the ability to grow on aërobic blood agar. Since this adaptation must have been accomplished through the acquisition of new respiratory systems, it was thought desirable to determine whether a change in

[10] Lockwood, J. S., *J. Immunol.*, 1938, **35**, 155.

drug susceptibility had likewise occurred. Therefore, the mouse tests were repeated using aërobic broth cultures as inoculum. Mouse-virulence was again attained with considerable difficulty and each strain tested in 30 mice receiving 20 mg of sulfanilamide per day and approximately the same number of lethal doses of culture as in Table I. No animal survived beyond the sixth day. Infection with strain S, which previously had been resistant to therapy, was now slightly less refractory. On the other hand infection with the 1097 strain, moderately susceptible when produced by the anaërobic variant, became definitely more resistant, so that the results with both strains were strikingly similar. It would appear, then, that the inability of streptococci to grow on aërobic blood agar may not necessarily be correlated with drug resistance in mice. In fact, strain 1097 was more susceptible to sulfanilamide when it was an obligate anaërobe.

Discussion. Bliss, Long and Feinstone[8] record anaërobic non-hemolytic streptococci as refractory to sulfanilamide both *in vitro* and *in vivo*. This opinion is in agreement with the clinical experience of Colebrook and Purdie.[9] The results with strain 1097 may be of interest, then, by suggesting the possibility that at least some strains of anaërobic hemolytic streptococci are amenable to sulfanilamide therapy.

The *in vitro* experiments of Shinn, *et al.*,[4] with Type I pneumococcus show that the gradual reduction of the oxygen concentration to 0.04% is accompanied by a corresponding decrease in bacteriostasis by sulfanilamide. With further reduction of oxygen, however, growth inhibition reappeared. It is possible that a similar mechanism is operating in our tests with the 1097 strain. By the same token strain S may possess a different respiratory mechanism making it resistant to the drug.

Summary. 1. Two weakly virulent strains of "anaërobic," Group A, hemolytic streptococci were subjected to *in vivo* and *in vitro* tests with sulfanilamide. 2. One strain was resistant, the other moderately susceptible, to the drug in mice. 3. No essential difference between the strains could be demonstrated, however, by *in vitro* bacteriostatic, phagocytic and biochemical tests. 4. Following adaptation to aërobic incubation (14 and 18 months) both strains were refractory in mice. 5. The results indicate that anaërobiosis, *per se*, was not the fundamental factor in determining drug response of these "anaërobic" hemolytic streptococci.

8 Bliss, E. A., Long, P. H., and Feinstone, W. H., *So. Med. J.*, 1938, **31**, 303.

9 Colebrook, L., and Purdie, A. W., *Lancet*, 1937, **2**, 1237.