serum, (3) incubation at body temperature, (4) various dilutions of the sera and (5) comparative effect of these sera on culture forms of *Leishmania tropica* and *Trypanosoma lewisi.*

Fresh serum from infected animals caused lysis of *Trypanosoma cruzi* taken from culture. When used in full strength, serum from blocked and infected animals caused lysis in a mean time of 88 min; while serum of control animals (which were infected only), caused lysis in 61.3 min, and the serum from uninfected animals which were blocked only, produced lysis in 153.6 min. Lysis never occurred in the physiologic saline controls, in which the organisms were mildly active even after 18 hr of observation. The serum of the animals subjected to mechanical block appeared to react more slowly. When serum had been inactivated at 56°C the organisms were immobilized by it, but lysis did not occur within the period of observation. When the preparations were kept at 37°C there was no significant change in reaction time from that at room temperature, although the reaction of the sera of the 2 infected groups of animals was slightly faster. No change at all was observed with serum from blocked uninfected animals or the physiologic saline control.

On the whole the concentration of the lysin in the sera was very low. A 1:1 dilution of the serum from animals which had been infected only caused lysis within 30 min to 1½ hr; serum from animals which had been blocked and infected, within 5 to 7 hr. A 1:1 dilution of the serum from animals which had been blocked only caused no lysis of the organism within the period of observation. A 1:5 dilution of serum produced lysis within 3 to 5 hr when taken from animals which were infected only, but required 7 or more hr when taken from those which were both infected and blocked. Serum of all 3 classes failed to produce lysis within 7 hr when a 1:10 dilution was used.

In a short series of tests using *Trypanosoma lewisi* and *Leishmania tropica* as test organisms, lysis did not occur but the motility of the organisms was rapidly impaired. The physiologic saline (control) as well as serum from all 3 groups of animals caused loss of motility of *L. tropica;* for *T. lewisi* only serum from infected and infected and blocked animals caused loss of motility of the organism.

Summary. Blood serum of animals infected with *Trypanosoma cruzi* caused lysis of this organism from cultures in less than 2 hr and, when diluted 1:5, in 3 to 5 hr. When the animals were blocked as well, the time required for lysis was increased and the lytic titer of the serum was reduced.

14010

Nature of the Activity of Sulfonamides for the Tubercle Bacillus.

ROBERT J. FITZGERALD AND W. HARRY FEINSTONE. (Introduced by G. W. Clark.)

From the Research Laboratories of the American Cyanamid Company, Stamford, Conn.

The failure of the sulfonamides to meet with any marked clinical success in the treatment of tuberculosis gives rise to speculation regarding the mode of action of the sulfonamides against the tubercle bacillus. A comparison of the *in vitro* effects of a number of sulfonamides against *Mycobacterium tuberculosis* and *Escherichia coli* indicates that the mode of action is probably identical in both cases.

Experimental. The bacteriostatic properties of a number of sulfonamides were tested against a laboratory strain of *E. coli* and a rapidly growing, non-pathogenic strain of *M. tuberculosis var. hominis* (American Type Culture Collection No. 607). For the *E. coli* tests, the compounds were serially diluted starting with 10 mg % and decreasing by half, *i.e.*, 10, 5, 2.5, etc. Each tube contained a final volume of 10 cc of syn-

TABLE I.
Acid Dissociation Values and Bacteriostatic Effect of Sulfonamides on *E. coli* and *M. tuberculosis*.

No.	Compound	pK_a*	*E. coli* min. effective conc. mols/L	*M. tuberculosis* min. effective conc. mols/L	Sulfanilamide coef.† for *E. coli*	Sulfanilamide coef.† for *M. tuberculosis*
1	Sulfanilamide	10.43	2.1x10−4	1.4x10−4	1.0	1.0
2	N1-Methyl sulfanilamide	10.77	2.7x10−4	1.3x10−4	0.78	1.07
3	N1-Phenyl "	9.60	2.5x10−5	1.0x10−4	8.4	1.4
4	N1-p-Tolyl "	9.82	4.7x10−5	1.3x10−4	4.36	1.07
5	N1-m-Tolyl "	9.74	4.7x10−5	1.3x10−4	4.36	1.07
6	N1-o-Tolyl "	9.96	9.5x10−5	2.6x10−4	2.25	5.4
7	Sulfapyridine	8.43	1.2x10−5	1.2x10−5	17.5	11.6
8	4-Sulfanilamido-pyridine	8.00	5.0x10−5	2.5x10−5	4.2	5.2
9	2-Sulfanilamido-5-amino pyridine	8.47	5.9x10−6	4.7x10−5	35.6	2.9
10	Sulfadiazine	6.48	1.0x10−6	1.5x10−6	210.0	93.5
11	2-Sulfanilamido-imidazole	9.60	4.2x10−4	4.2x10−4	0.5	0.33
12	Sulfapyrazine	6.04	1.5x10−6	1.5x10−6	140.0	93.5
13	Sulfathiazole	7.12	1.0x10−6	1.5x10−6	210.0	93.5
14	2-Sulfanilamido-1,3,4-thiadiazole	4.77	6.0x10−6	6.0x10−6	35.0	23.4
15	2-Sulfanilamido-5-methyl-1,3,4-thiadiazole	5.45	1.5x10−6	2.8x10−6	140.0	50.0
16	3-Sulfanilamido-5-methyl-1,2,4-oxadiazole	4.40	2.4x10−5	6.0x10−5	8.5	2.3
17	Sulfanilyl cyanamide	2.92	1.0x10−3	4.6x10−4	0.21	0.3
18	N1-Chloracetyl sulfanilamide	3.79	1.0x10−4	1.0x10−4	2.1	1.4
19	p,p′-Diamino diphenyl sulfone	—	2.5x10−5	6.2x10−6	8.8	22.6

*$pK_a = \log \frac{1}{K_a}$

†Sulfanilamide coefficient $= \frac{\text{Minimum effective conc. (sulfanilamide)}}{\text{Minimum effective conc. compound}}$

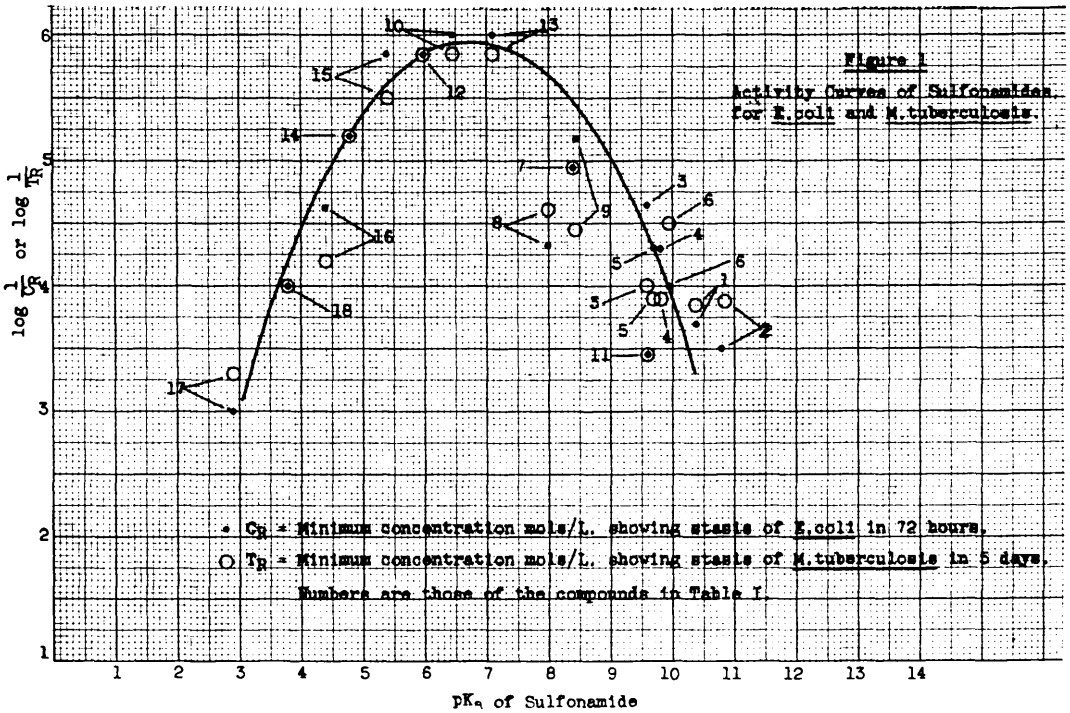
thetic, inhibitor-free medium as described by MacLeod.[1] The inoculum was so designed that each tube contained approximately 100 organisms per cc initially. Presence of growth was indicated by turbidity and the lowest concentration at which stasis continued for 72 hr was taken as the minimum effective concentration.

The same drug dilution scheme was employed for *M. tuberculosis*, but Dorset's synthetic medium was used as the substrate. Test tubes with a diameter of one inch were used to provide adequate surface area for the formation of the typical pellicle of the tubercle bacillus. Appearance of this pellicle was taken as the criterion of growth, and the highest dilution at which pellicle-formation failed to take place after 5 days was considered the minimum effective concentration. The inoculum for each tube was a finely divided saline suspension containing 0.1 mg moist weight of *M. tuberculosis* No. 607. Numerous repetitions have shown that these tests are reproducible to within plus or minus one drug dilution.

Results of these tests are listed in Table I. The minimum effective concentrations of the various sulfonamides are listed for both *E. coli* and *M. tuberculosis*. Also included are the acid dissociation constants for these compounds as determined by Bell and Roblin.[2] It will be noted that the compounds which have the greatest activity against *E. coli* display a similar effect on the tubercle bacillus, and compounds with a lesser degree of activity for the colon bacillus have a correspondingly diminished effect on *M. tuberculosis*. While it might be expected that the relative order of effectiveness of the compounds would be similar for the two organisms, it is probably fortuitous that the minimum effective concentrations fall so closely together in this case. Fig. 1 graph-

[1] MacLeod, C. M., *J. Exp. Med.*, 1940, **72**, 217.

[2] Bell, P., and Roblin, R., *J. A. C. S.*, 1942, **64**, 2905.



ically illustrates the relationship between the acid dissociation constants of the sulfonamides and their activity for the two organisms. The same type of activity curve is obtained in each case.

The effect of p-aminobenzoic acid on the tuberculo-static activity of several sulfonamides was studied. Varying amounts of p-aminobenzoic acid were incorporated in Dorset's medium containing varying amounts of the sulfonamides designed to cover their normal bacteriostatic range. Sodium 2,3,5-triiodobenzoate has been shown by Saz and Bernheim[3] to have some bacteriostatic activity for *M. tuberculosis*. This compound was also included here to see if p-aminobenzoic acid would have any effect on its activity. Results of this experiment are shown in Table II. The points at which growth is half inhibited are expressed as molecular ratios of the amounts of sulfonamide and p-aminobenzoic acid needed to bring about this condition. These ratios follow approximately the same order as those given by Wood,[4] Rubbo and Gillespie,[5] Rose and Fox,[6] and Wyss *et al.*[7] for *E. coli* and other organisms.

The effect of the sulfonamides on the tubercle bacillus is bacteriostatic rather than bactericidal. This could be demonstrated by the appearance of normal growth following addition of p-aminobenzoic acid to cultures that had previously undergone stasis for some time.

Discussion. It becomes apparent, from the experiments described in this paper, that compounds with a high antibacterial activity for *E. coli* may be expected to have a correspondingly high activity against *M. tuberculosis*, and the relative effectiveness is also similar for compounds which have an intermediate or low degree of activity against the colon bacillus. That is to say, while the minimum effective concentrations of the com-

[3] Saz, A. K., and Bernheim, F., *J. Pharm. and Exp. Ther.* 1941, **73**, 78.

[4] Wood, W. B., Jr., *J. Exp. Med.*, 1942, **75**, 369.

[5] Rubbo, S. D., and Gillespie, J. N., *Nature*, 1940, **146**, 838.

[6] Rose, H. M., and Fox, C. L., *Science*, 1942, **95**, 412.

[7] Wyss, O., Grubaugh, K. K., and Schmelkes, F. C., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 618.

TABLE II.

Effect of p-Aminobenzoic Acid on Antibacterial Action of Sulfonamides on *M. tuberculosis.*

Compound	Min. effective conc. mols per liter	Sulfanilamide coef.	R_p*	R_s†
Sulfanilamide	1.4×10^{-4}	1.0	16,600	1.0
Sulfapyridine	1.2×10^{-5}	11.6	2,800	5.9
p,p'-Diaminodiphenyl sulfone	6.2×10^{-6}	22.6	332	50.0
Sulfadiazine	1.5×10^{-6}	93.5	143	116.0
Sulfathiazole	1.5×10^{-6}	93.5	106	157.0
Sodium-2,3,5-triiodobenzoate	3.8×10^{-4}	0.36	PAB has no apparent effect	

*R_p = Molecular ratio between sulfonamide and p-aminobenzoic acid at point where antisulfonamide activity of p-aminobenzoic acid is half inhibited.

†$R_s = \frac{R_p \text{ for sulfanilamide}}{R_p \text{ for each sulfonamide}}$

pounds may not necessarily be identical, the relative *order* of effectiveness of a given group of sulfonamides will be similar. The observation of Bell and Roblin[5] that the potential bacteriostatic activity of a sulfonamide may be correlated with its acid dissociation constant is confirmed.

It has been shown that p-aminobenzoic acid is able to inhibit the bacteriostatic effect of the sulfonamides on *M. tuberculosis.* The amount of p-aminobenzoic acid necessary to bring about such inhibition increases with the relative bacteriostatic power of the individual sulfonamide. This would indicate that the mode of action of the sulfonamides for the tubercle bacillus is the same as for other microörganisms. These findings constitute further evidence in support of the contention of Wyss *et al.*[7] that the antibacterial activities of the sulfonamides are nonspecific.

While results obtained with a non-pathogenic strain may not necessarily hold true for virulent strains of the tubercle bacillus, it is felt that such confirmation will not be lacking. The work of Smith *et al.*,[8] and the results of experiments not yet published by the present authors, indicate that the sulfonamides that have been tested follow the same general order of effectiveness for the pathogenic as for the non-pathogenic strains of *M. tuberculosis,* though the minimum effective concentrations may be somewhat higher.

The use of the non-pathogenic strain provides a rapid and simplified quantitative bacteriostatic test for *M. tuberculosis* that is especially helpful in selecting active members from large groups of compounds.

The sulfonamides have been shown to be potentially as effective against the tubercle bacillus as they are against other microorganisms. It is not inconceivable, with the advent of inherently more active compounds or the discovery of more effective means to insure that the drugs reach the organisms, that the clinical response to tubercular infections will be more marked than can be demonstrated with the sulfonamides at present.

Summary. 1. A simple method for quantitatively estimating the effect of sulfonamides on the tubercle bacillus is described. 2. The antibacterial activity of the sulfonamides for *M. tuberculosis* can be correlated with their acid dissociation constants and their ability to withstand the inhibitory action of p-aminobenzoic acid. 3. The theories of the non-specificity of action of the sulfonamides may be extended to include their effect on the tubercle bacillus.

[8] Smith, M. I., *et al., J. Pharm. and Exp. Ther.,* 1942, **74**, 163.