From Fig. 1 A, it is apparent that in $[H^+]$ ranges where equation 2 applies, the predicted straight line plot is obtained, whereas in Fig. 1 B, it is shown that due to its high dissociation constant the sulfathiazole curve bends in accordance with equation 1. Moreover, the predicted relation between slope and K_S obtains.

As shown in Fig. 1 B and in accordance with equation 1 the increase of the efficiency of sulfathiazole with pH becomes progressively smaller, beginning approximately at pH 7. On the other hand, the corresponding point of inflection for sulfanilamide would be in the neighborhood of pH 10.5. If the pH is gradually increased above a value of 7, sulfanilamide should approach sulfathiazole in effectiveness. Preliminary experiments with organisms which grow in synthetic media buffered at slightly above pH 9 indicate that sulfanilamide actually approaches sulfathiazole in efficiency when this high pH is maintained.

Discussion. It should be borne in mind that while the degree of acidic dissociation governs the effectiveness of the active sulfonamides studied, other important factors are involved in the mechanism of sulfonamide action.

Further, this is only one aspect of the clinical problem which is complicated by considerations of absorption, solubility, toxicity, excretion, etc. Nevertheless, it would appear highly advantageous to maintain the pH of such infected loci that are to be treated with sulfonamides at the highest physiologically acceptable value. At pH 9 there is only comparatively little difference in the bactericidal effect of sulfanilamide and sulfathiazole.

Summary. From the nature of the function relating the effectiveness of sulfonamides to the hydrogen ion concentration the conclusion can be drawn that the active agent in a sulfonamide solution is an anionic species.

13728

Factors Influencing the Choice of Media for *in vitro* Sulfonamide Studies.

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Certain media are reported to inhibit the action of sulfonamides

in vitro. Lockwood[1] found that peptone inhibited the bacteriostatic and bactericidal action of sulfanilamide. MacLeod[2] has used a synthetic, inhibitor-free medium. A comparison of viable bacterial counts and bacteriostatic and bactericidal concentrations of sodium sulfathiazole in several of our media indicated that there was a direct relation between the nutritive quality of the media and the bacteriostatic and bactericidal concentrations of sodium sulfathiazole in the same media. In studying the growth curves of *Shigella paradysenteriae* Flexner in several media which we considered using for *in vitro* studies with sulfonamides, we observed (Table I) that certain media favored the development of a large bacterial population while other media supported the growth of only a small bacterial population. The synthetic medium[2] was composed of a mixture of salts with asparagine and glucose. This medium gave the least growth. Next in order were the nutrient broth, Bacto, which contained beef extract and peptone; and the 5% veal infusion, also a Bacto medium. The synthetic medium was much improved by the addition of 2% Witte's peptone. The brain-heart infusion medium Bacto, containing infusions from calf brains and beef heart together with proteose peptone and glucose, gave very much better growth than any of the others. Sodium sulfathiazole was added to these media in the following concentrations, 0.01 mg %, 0.1, 1.0, 5, 10, and increasing by 10 mg up to 100 mg %, then by 20 mg up to 200 mg %. When these media were inoculated with a few microörganisms we observed (Table I) that the medium which might be considered most inhibitory to the action of this sulfonamide was the medium which was most favorable to the development of the largest bacterial population, while the medium which appeared to be the least inhibitory was the one which was least favorable for an increase in population. In Table I are recorded the bacteriostatic and bactericidal concentrations of sodium sulfathiazole for *Shigella paradysenteriae* Flexner on 1, 2, 7, 14, and 21 days after inoculation of the various media with a small number of bacteria per cc. All media were incubated at 37°C for 4 weeks. Prolonged incubation resulted in increases in the bacteriostatic concentrations which in some instances occurred gradually and in others abruptly. When growth once appeared subcultures always remained positive for from 4 to 6 weeks. Viable bacterial counts during prolonged incubation in concentrations of sulfonamide which did not permit immediate growth showed that the original number of bacteria inoculated decreased

[1] Lockwood, J. S., and Lynch, Helen M., *J. A. M. A.*, 1940, **114**, 955.

[2] MacLeod, Colin M., *J. Exp. Med.*, 1940, **72**, 217.

TABLE I.
Effect of Quality of Medium, Bacterial Population and Time of Observation on Bacteriostatic and Bactericidal Concentrations of Sodium Sulfathiazole *in vitro*.

Media	Max.† viable counts‡ millions	Inocula per cc	Bacteriostatic conc. Days and mg % 1	2	7	14	21	Bactericidal conc. Days and mg % 1	2	7	14	21
Synthetic	10	46	*	*	*	*	*	*	*	*	*	*
Nutrient Broth	220	25	0.1	0.1	0.1	20	60	250	100	100	100	100
Veal Infusion	275	46	0.1	0.1	1.0	1.0	80	>150	150	80	80	80
Syn. + 2% Peptone	660	46	5.0	40	100	100	100	>400	400	100	100	100
Brain Heart Inf.	2080	25	<2.0	60	150	150	200	>200	>200	200	200	200

*No growth. Much larger inoculum necessary to obtain growth.
†Inoculum of 85 bacteria per cc in logarithmic phase.
‡Viable counts made after 12, 16, 20, 24, 30, 36 and 48 hours.

for a period of about 3 days and then increased, slowly at first, then rapidly, to a total number normal for that medium without sulfonamide. Growth became visible when the viable count was 15 to 20 million per cc.

Such late growth is suggestive of adaptation to environment. We have some data indicating that this may lead to the development of drug resistance *in vitro*. Such slow adaptation with its resultant increase in bacteriostatic concentration was observed in all media tested by us. The prolonged incubation would seem to introduce a factor which does not enter into the interesting report of Rose and Fox[3] which was published just as this paper was completed. They conclude that bacteria possess the ability to undergo only a certain limited number of cell divisions in the presence of a bacteriostatic concentration of a sulfonamide, regardless of the size of the inoculum. Their time of observation was only 4 days. Our results would indicate that a certain concentration of sulfonamide may be bacteriostatic for as long as 7 days (0.1 mg % in the case of nutrient broth, Table I) after which time growth occurred not only in the concentration which had been bacteriostatic for 7 days, but also in higher concentrations.

Frequent subcultures were made of each of the 19 different concentrations of drug to determine the bactericidal concentration of the sulfonamide in each medium. The bactericidal concentrations were high at first but decreased rapidly and usually within 7 days reached a level ultimately shown to be the maximum bacteriostatic concentration. It is observed in Table I that brain-heart infusion medium, which favored a high bacterial count, showed a bacteriostatic concentration on the second day of 60 mg % and on the 21st day 200 mg %. The bactericidal concentration was 200 mg %. Nutrient broth

[3] Rose, H. M., and Fox, Charles L., *Science*, 1942, **95**, 412.

which gave next to the lowest bacterial count showed a bacteriostatic concentration for the same microörganism on the second day of 0.1 mg %, and on the 21st day 60 mg %. The final bactericidal concentration in this medium was 100 mg %. It would seem, therefore, that values for the bacteriostatic and bactericidal concentrations of a sulfonamide compound for a certain microörganism *in vitro* are dependent upon certain variables such as nutritive quality of medium and time of observation.

A third factor influencing the bacteriostatic and bactericidal concentrations is the number of bacteria inoculated into the medium. The influence of this factor is demonstrated in Table II, where the inoculum for nutrient broth varied from 33 to 33,000 bacteria per cc. The results observed in Table II show that the bacteriostatic and bactericidal concentrations of sodium sulfathiazole and sulfaguanidine increased as the number of bacteria in the inocula were increased. For instance, the bacteriostatic concentration of sodium sulfathiazole was 1 mg % on the seventh day when the inoculum was 33 bacteria per cc, and 80 mg % on the same day with an inoculum of 33,000 bacteria per cc. The bactericidal concentration on the seventh day was 1 mg % with an inoculum of 33 bacteria per cc, and 100 mg % when the inoculum was 33,000 bacteria per cc. In general, the time of observation was again a factor as indicated by increasing bacteriostatic concentrations and decreasing bactericidal concentrations with continued incubation.

The same 3 factors exerted similar influences when sulfaguanidine was used but it is observed in Table II that this sulfonamide was much less bacteriostatic and bactericidal. The highest concen-

TABLE II.
Influence of Number of Bacteria in Inoculum.

Inocula *Shig. Para.* Flexner per cc	Sodium sulfathiazole in nutrient broth Days and mg%							
	Bacteriostatic conc.				Bactericidal conc.			
	2	7	14	21	2	7	14	21
33	1	1	1	1	10	1	1	1
330	1	1	1	10	20	10	10	10
3300	1	1	40	40	150	60	60	60
33000	1	80	80	100	200	100	100	100
	Sulfaguanidine in nutrient broth							
33	10	20	20	20	>200*	20	20	20
330	20	80	>200*	>200*	>200*	>200*	>200*	>200*
3300	40	>200*	>200*	>200*	>200*	>200*	>200*	>200*
33000	80	>200*	>200*	>200*	>200*	>200*	>200*	>200*

*Highest concentration used and not sufficient to give ultimate bacteriostatic and bactericidal effect with prolonged incubation.

tration of sulfaguanidine used in these experiments was 200 mg %, since solubility is only 220 mg %.

Summary. Studies of the effect of certain media on the action of sulfonamides indicate that a medium which may seem to be inhibitory is one which favors the growth and multiplication of bacteria so that an ultimate large bacterial population results. Our data also indicate that a medium, which by comparison may seem not to be inhibitory, is one which is not favorable to the development of large numbers of the bacteria under study. It is also apparent that the type of medium, time of incubation and number of bacteria inoculated are important factors which should influence conclusions drawn from sulfonamide studies *in vitro*. Prolonged incubation may result in adaptation of the bacteria to unusually high concentrations of the sulfonamide.

13729

Inhibition of Antimalarial Action of Sulfonamides by p-Aminobenzoic Acid.

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It has been found that p-aminobenzoic acid prevents the inhibitory action of sulfanilamide on the growth of hemolytic streptococci *in vitro*[1, 2] and *in vivo*.[3] This compound also inhibits the therapeutic effect of sulfanilamide on mice inoculated intracerebrally with the virus of lymphogranuloma venereum.[4] It has been suggested[2, 4] that sulfanilamide exerts its action by competing for an enzyme associated with a metabolite similar in structure to p-aminobenzoic acid and essential to the organism.

Coggeshall[5] showed that sulfanilamide had a marked effect against plasmodia (*Plasmodium knowlesi* in rhesus monkeys). Other sulfonamides[6] were shown to be effective against *P. knowlesi* and against *Plasmodium cynomolgi* and *Plasmodium inui* in rhesus monkeys.

1 Woods, D. D., and Fildes, P., *Chem. and Ind.*, 1940, **59**, 133.

2 Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

3 Selbie, F. R., *Brit. J. Exp. Path.*, 1940, **21**, 90.

4 Findlay, G. M., *Brit. J. Exp. Path.*, 1940, **21**, 356.

5 Coggeshall, L. T., *Am. J. Trop. Med.*, 1938, **18**, 715.

6 Coggeshall, L. T., and Maier, J., *J. Inf. Dis.*, 1941, **69**, 108.