Conclusions. (1) A 3-day curative technic for the biological assay of the antihemorrhagic factor has been developed. If a vitamin K standard is run with each group of chicks and certain precautions in the procedure are observed, the assay is quantitative within the limits of error for a biological assay. (2) The vitamin K activity of a compound varies significantly with the solvent and the method of administration. (3) The vitamin K activity of 2-methyl-1,4-naphthoquinone and of 2-methyl-1,4-naphthoquinhydrone is approximately the same as the natural vitamin K_1 on a molar basis.

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Action of Sulfanilamide on Hemolytic Enterococcus (*Streptococcus fecalis hemolyticus*).

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It is generally agreed that sulfanilamide, sulfapyridine, and related compounds are effective in the treatment of infections due to a limited number of microörganisms only. *In vitro,* these chemotherapeutic substances may be bacteriostatic or bactericidal toward susceptible strains. There is, however, no absolute parallelism between the effectiveness of sulfanilamide *in vivo* and *in vitro;* nor between susceptibility or resistance of certain species or groups of microörganisms and their respective position in the system of bacteria. The susceptibility toward the action of sulfanilamide may be quite different even among relatively closely related groups. Thus, sulfanilamide may inhibit the growth of Lancefield Group A hemolytic streptococci in broth, while strains of hemolytic streptococci Group D (hemolytic enterococci) are quite resistant toward this drug (Bliss and Long[1]). Recently, it was reported[2, 3] that sulfanilamide in a concentration of 800 mg per 100 ml may continuously inhibit the growth of fibrinolytic hemolytic streptococci even when relatively large numbers were used for inoculation; in contrast, strains of hemolytic enterococci, characterized by their growth on 40% bile agar, by their capacity to reduce methylene blue and litmus in

1 Bliss, S. A., and Long, P. H., *New England J. Med.,* 1937, **217**, 18.

2 Neter, E., *J. Bact.,* 1938, **36**, 669.

3 Neter, E., *J. Lab. and Clin. Med.,* 1939, **24**, 650.

skimmed milk, by their production of a pH below 4.8 in 1% glucose broth, and by their ability to ferment mannitol, were only slightly or not at all inhibited in 0.8% sulfanilamide broth. The culture medium used in these experiments was phenol-red broth (Difco), to which 1% soluble starch, 0.2% agar, and 1% maltose or dextrose, respectively, were added. Subsequently, it was found that essentially the same results were obtained when starch and agar were omitted from this medium. These observations are in agreement with the findings of Long and Bliss,[4] who showed that streptococci of Group D are resistant toward the bacteriostatic action of sulfanilamide in concentrations up to 0.8%; while in higher concentrations this drug slightly retarded the growth of a few strains. It was noted that the strains of hemolytic enterococci at our disposal grew more rapidly in the above described phenol-red broth than did Group A hemolytic streptococci; visible growth occurred within a few hours following inoculation of the medium. Observations[5–10] of the so-called "lag-period" in the action of sulfanilamide and sulfapyridine, both *in vivo* and *vitro,* suggested that the rapid growth of hemolytic enterococci may in part be responsible for the negligible bacteriostatic action of sulfanilamide toward these strains under the above-reported conditions. Sherman[11] showed that both hemolytic enterococci *(Streptococcus zymogenes* and *Streptococcus durans)* and non-hemolytic enterococci *(Streptococcus fecalis* and *Streptococcus liquefaciens)* may grow in broth containing 6.5% sodium chloride in contradistinction to hemolytic streptococci Groups A, B, and C. Employing this test for differential diagnostic purposes, it was noted that the growth of hemolytic enterococci in this medium was much slower than that in broth containing only 0.5% sodium chloride. This retarding influence of sodium chloride on the growth of hemolytic enterococci was utilized in the following experiments dealing with the action of sulfanilamide on hemolytic enterococci in broth containing 6.5% sodium chloride.

The medium was prepared as follows: Phenol-red broth (Difco) containing tryptose, 0.5% sodium chloride, 0.1% dipotassium phos-

[4] Long, P. H., and Bliss, E. A., *The Clinical and Experimental Use of Sulfanilamide, Sulfapyridine, and Allied Compounds,* The Macmillan Company, 1939, 102.

[5] Long, P. H., and Bliss, E. A., *J. Am. Med. Assn.,* 1937, **108**, 32.

[6] Buttle, G. A. H., *Proc. Roy. Soc. Med.,* 1937, **31**, 1540.

[7] Osgood, E. E., *J. Am. Med. Assn.,* 1938, **110**, 349.

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

[9] Whitby, L., *Lancet,* 1938, **2**, 1095.

[10] McIntosh, J., and Whitby, L., *Lancet,* 1939, **1**, 431.

[11] Sherman, J. M., *Bact. Rev.,* 1937, **1**, 16.

phate, and phenol red as indicator was dissolved in the given amount in neutral distilled water; then the respective amounts of maltose and sodium chloride were added. Sulfanilamide (purified) in the amount of 0.8% was added to one part of this medium, while the other part was used as control. The broths were autoclaved at 15 pounds pressure for 12 minutes; the medium was used only if no noticeable change in the pH occurred following this procedure.

Four strains of hemolytic enterococci were used. Two of these were obtained through the courtesy of Dr. J. M. Sherman, Ithaca, New York. For inoculation, 0.1 cc of a 2- to 5-hour brain-heart-infusion broth-culture or dilution thereof, was added to about 5 cc of each sulfanilamide and control broth. The tubes were incubated at 37°C; growth and acid-production were noted at various intervals.

Table I presents the results of an experiment on the bacteriostatic action of sulfanilamide (0.8%) on a strain of hemolytic streptococcus Group A and a strain of hemolytic enterococcus (Group D) in 1% maltose phenol-red broth containing 0.5% and 6.5% sodium chloride, respectively. It may be seen, that: (1) sulfanilamide in broth containing 0.5% sodium chloride retarded the growth of the hemolytic enterococcus only to a slight degree, while it completely and continuously inhibited the growth of Group A hemolytic streptococcus; (2) the growth of the hemolytic enterococcus was markedly delayed in broth containing 6.5% sodium chloride, while Group A hemolytic streptococcus failed to grow; (3) sulfanilamide (0.8%) in broth containing 6.5% sodium chloride prevented visible growth of the hemolytic enterococcus for 2 days and later inhibited the resulting growth. This bacteriostatic action of sulfanilamide on hemolytic enterococcus was repeatedly demonstrated with 4 strains at our disposal—provided that a suitable number of bacteria were used for inoculation. In a number of experiments, sulfanilamide in concentration of 1% completely prevented visible growth of the hemolytic enterococcus, even when incubation was carried out for one week. It must be emphasized, however, that the results of these experiments on the bacteriostatic action of sulfanilamide on hemolytic enterococci were not as striking and uniform as those obtained with fibrinolytic hemolytic streptococci.

Preliminary experiments indicate that the degree of effectiveness of sulfanilamide on the growth of hemolytic enterococci is linked to the carbohydrate-content of the medium. Thus, in the presence of 1% dextrose, the bacteriostatic action of sulfanilamide toward these strains was negligible. On the other hand, when the maltose-content of the medium was reduced from 1% to ¼%, sulfanilamide in a concentration of 1% definitely inhibited the growth of small numbers of

TABLE I.

Bacteriostatic Action of Sulfanilamide on *Streptococcus hemolyticus* and *Enterococcus hemolyticus*

Hrs Incubation 37°C	Growth and acid-production by I *Streptococcus hemolyticus* in phenol-red maltose broth containing				Growth and acid-production by II *Enterococcus hemolyticus* in phenol-red maltose broth containing			
	a .5% NaCl	b .5% NaCl + .8% Sulfanilamide	c 6.5% NaCl	d 6.5% NaCl + .8% Sulfanilamide	a .5% NaCl	b .5% NaCl + .8% Sulfanilamide	c 6.5% NaCl	d 6.5% NaCl + .8% Sulfanilamide
2	+	—	—	—	+	—	—	—
8	+++	—	—	—	++++	+++	+	—
18	++++	—	—	—	++++	+++	++	—
48	++++	—	—	—	++++	+++	+++	—
72	++++	—	—	—	++++	+++	+++	+

— = no growth and no acid-production.

+ to ++++ = various degrees of growth and acid-production.

hemolytic enterococcus, even when the sodium-chloride content of the medium was only 0.5%.

The demonstration of the bacteriostatic action of sulfanilamide in high concentrations on hemolytic enterococci in phenol-red broth containing from 6.5 to 7% sodium chloride, may possibly be explained on the basis of Mellon's[12] observation of a potentiating effect of sodium chloride and sulfanilamide. Or, the possibility may be considered that the retardation of growth by sodium chloride may overcome the lag-period necessary for the action of this drug; this latter point of view, however, leaves unexplained the fact that growth may occur after several days of incubation. The foregoing experiments make it seem possible that other changes in the environmental conditions may enhance the bacteriostatic action of sulfanilamide toward species and groups of microörganisms that thus far were considered to resist the action of the drug.

In conclusion: (1) Sulfanilamide in concentrations of 0.8% to 1% in 1% maltose phenol-red broth retarded the growth of hemolytic enterococci to a slight degree only, while it continuously inhibited the growth of fibrinolytic hemolytic streptococci. (2) A more marked bacteriostatic action of sulfanilamide (0.8 to 1%) on small numbers of hemolytic enterococci could be demonstrated when the sodium-chloride content of this medium was increased to 6.5% or 7%, or when the maltose-content was decreased from 1% to ¼%.

[12] Mellon, R. R., Gross, P., and Cooper, F. B., *Sulfanilamide Therapy of Bacterial Infections*, Charles C. Thomas, 1938, 233.