SPECIAL ARTICLES

THE BACTERIOSTATIC ACTION OF SULFANILAMIDE UNDER ANAEROBIC CONDITIONS

ALTHOUGH the therapeutic use of sulfanilamide and allied compounds has been attended with much clinical success, the mechanism of their action in overcoming various infections is still in dispute. The *in vitro* studies of Locke,[1] Fox and coworkers[2] and the theoretical deductions of Shaffer[3] have led many investigators to assume that sulfanilamide is bacteriostatic only in the presence of oxygen. Contrary to such claims are the important findings of Bliss and Long[4] who reported bacteriostasis of streptococci and pneumococci under anaerobic conditions. However, Fox[5] claimed that Bliss and Long had been unable to obtain complete anaerobiosis in their experiments and attributed these results to small amounts of oxygen still remaining in their media.

We wish to report some data which completely substantiate the claim of Bliss and Long that sulfanilamide may be bacteriostatic under anaerobic conditions. During the course of some experiments designed to test the validity of the reduction potential theory of Shaffer and of the anti-catalase hypothesis of Locke, we have had occasion to examine the effects of sulfanilamide on the growth of *E. coli*. This was done primarily because the respiratory mechanisms and nutritional requirements of this organism are better understood than are those of the coccus group and because it has been pointed out[6] that *E. coli* is an excellent test organism for the accumulation of hydrogen peroxide.

Sulfanilamide produces, under given circumstances, marked bacteriostasis in cultures of *E. coli*. Thus, in ordinary meat extract broth, bacteriostasis is observed under aerobic but not under anaerobic conditions. On the other hand, in broth containing 1 per cent. glucose, sulfanilamide produces but little effect. It might be claimed that this protective action of glucose is attributable to its powerful reducing properties. Such does not appear to be the case, however. Both cysteine and ascorbic acid, powerful reducing agents themselves, fail to protect against the sulfanilamide bacteriostasis. Furthermore, as will be reported in another paper, glucose does not protect against the activity of sulfanilamide towards *E. coli* in certain other media.

It appears more probable that the protective action of glucose is based on its ability to supply to the cell a source of food and energy that is unaffected by the presence of sulfanilamide. *E. coli,* when growing in nutrient broth, depends on the various constituents of this medium for its supply of food materials, and if sulfanilamide, for any reason whatsoever, prevents the assimilation of various of these the growth of the cells will be adversely affected. On the other hand, if another substance (glucose) assimilable in nutrient broth even in the presence of sulfanilamide is added to the basal medium, growth will not be affected to any noticeable degree provided that such a substance can supply energy sufficient to permit growth to the same degree as do the constituents of the basal medium.

The proposition that sulfanilamide inhibits the mechanisms which enable the organism to grow aerobically at the expense of the constituents of nutrient broth finds some support in the following facts (Table I). *E. coli* grows well aerobically in nutrient broth

TABLE I

THE EFFECT OF SULFANILAMIDE ON GROWTH OF *E. coli* IN NUTRIENT BROTHS

Oxygen tension	Nutrient broth enriched with			
	1 per cent. glucose			
	Sulfanilamide 10 mg per cent.	———	Sulfanilamide 10 mg per cent.	———
Aerobic	++++	++++	+	+++
Anaerobic	++++	++++	+	+

but only poorly under anaerobic conditions. In the presence of oxygen, the energy required for the multiplication of such a facultative anaerobe as *E. coli* is obtained simultaneously from two different sources—the one, an aerobic, the other an anaerobic mechanism. If sulfanilamide inhibits only the aerobic mechanism, the degree of growth in the presence of this chemical and oxygen should approximate that obtained in the absence of oxygen, and sulfanilamide should have no effect under conditions which induce anaerobiosis. Such is actually the case.

Moreover, since the addition of glucose enables the organisms to grow luxuriantly both in the presence and absence of oxygen, neither type of growth in glucose broths should be affected by sulfanilamide if the latter does not inhibit the assimilation of glucose. The results in Table I demonstrate that both aerobic and anaerobic growth in glucose broth are the same in the presence as in the absence of sulfanilamide.

[1] A. Locke, E. R. Main and R. R. Mellon, SCIENCE, 88: 620, 1938.

[2] C. L. Fox, B. German and C. A. Janeway, *Proc. Soc. Exp. Biol. Med.*, 40: 184, 1939.

[3] P. A. Shaffer, SCIENCE, 89: 547, 1939.

[4] E. Bliss and P. Long, *Proc. Third Internat. Microbiol. Cong.*, New York, 1939.

[5] C. L. Fox, *Proc. Third Internat. Microbiol. Cong.*, New York, 1939.

[6] R. H. Broh-Kahn and I. A. Mirsky, *Jour. Bact.*, 35: 455, 1938.

The type of bacteriostasis that has just been described is quite similar to that observed in the case of the hemolytic streptococci, since there is in both cases the same lag period before the bacteriostatic action becomes evident.

Quite different results were observed when the organism was grown in a synthetic medium of the following composition:

4.0 g. $(NH_4)_2HPO_4$	0.7 g. $MgSO_4$
1.0 g. NaCl	Trace $FeSO_4$
1.0 g. KH_2PO_4	1000 cc. distilled water

Adjust to pH 7

As has been demonstrated by Quastel *et al.*,[7] *E. coli* grows well aerobically in such a medium when 1 per cent. lactate is added, but not at all under anaerobic conditions. On the other hand, the addition of both 1 per cent. lactate and 1 per cent. nitrate enables the organisms to grow anaerobically as well as aerobically, since the cells can utilize the energy obtained from the oxidation of lactate by nitrate to multiply in the absence of oxygen.

When sulfanilamide is added to cultures growing aerobically in the lactate medium, very little bacteriostasis is evident. The same is true for aerobic cultures growing in the lactate-nitrate medium. However, anaerobic cultures in the latter medium are markedly inhibited by the action of sulfanilamide. This, then, is an example of a case in which sulfanilamide is bacteriostatic in anaerobic but not in aerobic environment.

It is apparent that sulfanilamide prevents the oxidation of lactate by nitrate and so exerts its effect in anaerobic cultures. In this respect its action is similar to that of the cyanides (Table II).

THE EFFECT OF SULFANILAMIDE AND CYANIDES ON THE GROWTH OF *E. coli* IN A SYNTHETIC MEDIUM

Oxygen tension	Synthetic medium + 1 per cent. Lactate enriched with					
			———	1 per cent. nitrate		
	Sulfanilamide 10 mg per cent.	KCN M/400	——	Sulfanilamide 10 mg per cent.	KCN M/400	——
Aerobic ..	++	++	++	+++	++	+++
Anaerobic .	—	—	—	—	—	+++

These results are not affected by the various methods employed to obtain anaerobiosis, and they have been observed under conditions in which the oxygen content is so low that it will not affect electrode potentials or reoxidize leuco dyes. The following biological criteria of anaerobiosis could be obtained under the conditions employed. It is known[8] that *E. coli* is extremely sensitive to oxygen when grown in media containing cyanides and certain reversible oxidation-reduction systems. Furthermore, the growth of *H. influenzae* in the absence of hemin is inhibited by the slightest traces of oxygen in the medium[9] and our methods of securing anaerobiosis were sufficient to permit the growth of both organisms under the conditions mentioned. Certainly, the few molecules of oxygen that remain in the medium are insufficient to produce enough peroxide, "blue substance"[10] or oxidized sulfanilamide to cause bacteriostasis.

These observations of the differences between the effects of sulfanilamide in the nutrient broths and in synthetic media suggest that sulfanilamide selectively inhibits only certain mechanism. It follows that if, perchance, such mechanisms are either essential or beneficial to multiplication, the growth of the organism will be inhibited to a greater or less extent.

The above results should not necessarily be construed to indicate that the theories of Locke, Shaffer and Fox may not explain the bacteriostasis observed *in vitro* under special conditions, but they appear to demonstrate that such mechanisms can not be the sole ones concerned. Experiments in this laboratory have led us to believe that the bacteriostasis produced by sulfanilamide and allied compounds can not be attributed to any general, non-specific mechanism and that all efforts to derive knowledge concerning its mode of action will be futile unless its effects are examined in a variety of media under different conditions.

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EFFECT OF NICOTINAMIDE ON RESPIRATION OF DYSENTERY BACILLI

NICOTINIC acid has been shown to be essential for the growth of the dysentery organism on a synthetic medium composed of amino acids, glucose and salts.[1] Derivatives of nicotinic acid such as the amide, coenzyme I and coenzyme II, and other closely related compounds will also function as growth factors in a synthetic medium.[2] On the other hand, Lwoff and Lwoff have shown[3] that *Hemophilus parainfluenzae*

[7] J. H. Quastel, M. Stephenson and M. Whetham, *Biochem. Jour.*, 19: 304, 1925.

[8] R. H. Broh-Kahn and I. A. Mirsky, *op. cit.*

[9] T. L. Snyder and R. H. Broh-Kahn, *Nature*, 142: 153, 1938.

[10] C. L. Fox, B. German and C. A. Janeway, *op. cit.*

[1] Koser, Dorfman and Saunders, *Proc. Soc. Exp. Biol. Med.*, 38: 311–13, 1938.

[2] Dorfman, Koser, Reames, Swingle and Saunders, *Jour. Infectious Diseases*, 65: 163–82, 1939.

[3] *Proc. Roy. Soc., London*, B122: 352–9, 1937.