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.

needs the entire molecule of coenzyme I or coenzyme II and can not synthesize it from nicotinamide. The dysentery organism when grown on a synthetic medium with nicotinamide can synthesize a compound (or compounds) identical with or an adequate substitute for one or more of the coenzymes needed by *H. influenzae*.[2]

By means of the Thünberg technique, Lwoff and Lwoff have shown[4] that *H. parainfluenzae* cells grown in a medium which is deficient in the "V" factor (presumably coenzyme I or coenzyme II) have their respiration markedly stimulated by the addition of coenzyme I or coenzyme II to the Thünberg tubes. A similar stimulation of oxygen uptake could be demonstrated by means of the Warburg technique. This stimulation disappears when the cells are grown in a medium containing larger amounts of "V" factor, *i.e.*, the addition of coenzymes to the Thünberg tubes does not increase the respiration.

We have performed similar experiments in which we used dysentery bacilli grown on a medium containing suboptimum amounts of nicotinamide. We found that the rate of reduction of methylene blue is greatly increased not only by the addition of coenzyme I, but also by the addition of nicotinamide or nicotinic acid to the Thünberg tube, as shown by the following protocol. This effect of nicotinamide and nicotinic acid has not previously been reported for any system. The cells (Strain D76) were grown 24 hours in synthetic medium containing 0.005γ nicotinamide. The cells from 100 cc of medium were collected in the centrifuge and suspended in M/20 buffer (pH 7.4). The cells were again centrifuged and suspended in 34 cc of buffer. Two cc of this suspension was added to each tube. Each tube also contained 0.30 cc of a 2 per cent. solution of glucose and 0.50 cc of a solution of methylene blue containing 100γ of methylene blue per cc. The nicotinamide and nicotinic acid solutions contained 100γ per cc. The cozymase solution contained 300γ per cc and was 40 per cent. pure. Smaller quantities of a pure cozymase preparation from Euler's laboratory showed the same effect. Water was added to the controls to make the dilution the same in all cases. The tubes were equilibrated 10 minutes at 37° before tipping.

TABLE 1

Tube	Control	1	2	3
Nicotinamide	0	0.15 cc	0	0
Nicotinic acid	0	0	0.15 cc	0
Cozymase	0	0	0	0.15 cc
Time (in sec.)	460	170	170	200

If we consider the ratio of the time of reduction control/nicotinamide, we find that the ratio decreases as the amount of nicotinamide in the *medium* is increased until with cells grown in a medium containing 0.020γ per cc it is about one. Thus at 0.003γ, the ratio is 3.8; at 0.005γ, 2.6; at 0.007γ, 1.8; at 0.010γ, 1.5; and at 0.020γ, 1.1. In controls containing nicotinamide, etc., but not glucose, reduction was much slower, 600 seconds or more. In controls which contained cells alone the methylene blue was reduced even more slowly or not at all. Nicotinamide, nicotinic acid or cozymase and glucose gave no reduction of methylene blue in the absence of cells.

The same stimulation of respiration by nicotinamide, etc., was found when oxygen uptake was measured by the direct Warburg method. It should be emphasized that various strains of the dysentery organism differ in their nicotinamide requirements. Further work in an attempt to elucidate the mechanism of this codehydrogenase action of nicotinamide is in progress in this laboratory.

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[4] *Ibid.*, 360–73.

SOMATOPLASTIC STERILITY IN MEDICAGO SATIVA[1]

THE collapse of ovules during the early stages of post-fertilization development frequently occurs in alfalfa, particularly after self-pollination.[2] Histological study shows that the collapse follows abnormal growth of the somatic tissue adjacent to the embryo sac. Since local hyperplasia of the maternal structures appears to be the essential feature of the developmental course leading to collapse, the term somatoplastic sterility is here proposed for a type of seed failure believed to occur in many plants.

The reproductive process in angiosperms may fail at any stage between fertilization and maturity of the seed. The early stages are of critical importance for survival of the ovule in alfalfa. Although somatoplastic sterility is not necessarily limited to this period, it is in young ovules that one would expect it to be manifested in simplest form. The case is of interest, therefore, as affording a point of departure in defining the significant histological features and in discovering the cause of a rather obscure, although probably widespread, form of sterility in plants.

The embryo sac in the mature ovule of alfalfa is surrounded by two integuments. The inner integument, which is composed of two layers of cells, lies in direct contact with the embryo sac except at the chalazal end where a few disintegrating cells, remnants of

[1] Papers from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin No. 248. The authors desire to express their appreciation for financial aid from the University Research Committee and for assistance from WPA project in the Natural Sciences No. 8649.

[2] Brink and Cooper, *Proc. Nat. Acad. Sci.*, 24: 497–499, 1938.